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(57) Abstract

Bipolar lipids are described which are able to form complexes with polyanions. The lipids comprise a cationic head linked to a hydrophobic backbone and a hydrophilic tail and are capable of self assembly to form stable complexes in aqueous solutions. The lipids are of particular use for the delivery of bioactive substances such as nucleic acids to cells *in vitro* and especially *in vivo*.

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BIPOLAR LIPIDS AND THEIR USE FOR THE DELIVERY OF BIOACTIVE SUBSTANCES

This invention relates to a series of bipolar lipids and to their use to deliver bioactive substances to cells.

To be effective, many pharmaceutical agents need to be efficiently delivered to the cytoplasm of a eucaryotic cell. For many low molecular weight compounds of low to moderate polarity this is not a problem since such molecules can pass directly through the plasma membrane of the cell and into the cytoplasm. Direct passage is not available to other compounds of greater polarity or high molecular weight and these generally enter the cell by receptor mediated endocytosis or phagocytosis. These mechanisms are not efficient however with all sizes and types of molecule. In particular, large, polyanionic compounds are not readily taken up by cells when delivered to them in aqueous solution.

One general solution to this problem is to couple any poorly transported pharmaceutical agent to a carrier which itself is readily taken up into the cytoplasm of a cell. This is not always satisfactory however, since coupling to the carrier may have an undesirable effect on the metabolism and/or antigenicity of the pharmaceutical agent and/or it may be difficult to recover the desired biological activity from the resulting conjugate once inside the cell.

An alternative solution is to formulate the pharmaceutical agent with a delivery vehicle which is soluble in aqueous solutions but which can also mimic naturally occurring cell membrane constituents. This encourages fusion of the vehicle with a cell membrane and subsequent delivery of any associated pharmaceutical agent to the cytoplasm.

Amphiphilic lipids have frequently been used for this purpose. These typically have a hydrophobic backbone composed of one or more hydrocarbons and a hydrophilic polar head group containing one or more ionisable groups, to facilitate the transport of macromolecules to and across the plasma membrane of cells and into the cytoplasm. The polarity of the head group may be controlled by the selection of the number and/or

type of ionisable groups to achieve a range of negatively charged (anionic), neutral or positively charged (cationic) lipids.

For the delivery of polyanions it is generally advantageous to use cationic lipids. The advent of gene therapy and the need to deliver anionic molecules such as nucleic acids to mammalian cells has provided much impetus to the development of this class of lipids. First generation compounds include those with a monocation head group such as N-[1(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride [DOTMA; Felgner, P L and Ringold, G M, Nature, 337 387-388 (1989)], 1,2-dimyristyloxypropyl-3-dimethylhydroxyethylammonium bromide [DMRIE; Zabner, J et al J. Biol. Chem, 270, 18997-19007 (1995)] and 3β[N-(N^1,N^1-dimethylamino-ethane)carbamoyl]cholesterol [DC-Chol; Farhood, H et al, Biochim. Biophys. Acta. 1111, 239-246 (1992)] and those with a polycation head group such as dioctadecyl-amidoglycylspermine [DOGS; Behr, J-P, et al, Proc. Natl. Acad. Sci. 86, 6982-6986 (1989)].

In an effort to improve the properties of these early compounds for in vivo delivery of polyanions many more cationic lipids have been developed in which the nature and size of the hydrophobic backbone and/or the cationic head group have been varied (see for example International Patent Specifications Nos. WO95/21931, WO96/10038, WO96/17823, WO96/18273, WO96/25508, WO96/26179, WO96/41606, WO97/18185, WO97/25339, WO97/3010 and WO97/31934).

The goal in the development of cationic lipids for in vivo use is to provide a molecule which is simple to use in a clinical setting; which is robust; which forms small stable complexes over wide pH and ionic strength ranges; which is non-toxic; and which is capable of delivering a high concentration of polyanion to a cell.

We have now developed a class of lipid which meets these requirements. Importantly, our lipids are capable of self-assembly and will form stable complexes in aqueous solutions. The lipids are able to efficiently compact polyanions to give defined particle sizes of less than 500nm. The lipid-polyanion complex remains associated over wide pH and ionic strength

ranges and is able to efficiently deliver high concentrations of polyanions to c lls.

Thus according to one aspect of the invention we provide a bipolar lipid comprising a cationic head (1) a hydrophobic backbone (2) and a hydrophilic tail (3) in which:

(A) the cationic head comprises two or more cationic centres, each centre containing at least one heteroatom and being covalently linked to one or more other centres by one or more carbon containing spacer groups;

(B) the hydrophobic backbone comprises one or more hydrocarbon chains; and

(C) the hydrophilic tail comprises one or more acyclic hydrophilic hydrocarbons each containing two or more atoms or groups capable of being solvated by water;

each of said components (1) to (3) being covalently linked head (1) to backbone (2) to tail (3) and arranged such that at least one hydrocarbon chain in the hydrophobic backbone (2) is covalently linked to a heteroatom in a cationic head (1) and each hydrophilic hydrocarbon in the hydrophilic tail (3) is covalently linked to a chain in the backbone (2) to achieve at least a ten atom spacing along the chain between the tail (3) and the head (1).

In the lipids according to the invention, each cationic centre in the cationic head (1) may be provided by one or more heteroatoms capable of retaining a positive charge at a pH in the range from around pH 2.0 to around pH 10.0. In practice, whether a heteroatom will retain a positive charge in this pH range will depend on the nature and number of any other atoms or groups attached to it. Thus particular examples of suitable cationic centres include primary, secondary, tertiary and quaternary amino groups, sulphonium and phosphonium groups.

The number of cationic centres may be varied as desired depending on the intended use of the lipid of the invention. At least two centres will be present, but three, four, five, six, seven, eight or more may be optionally incorporated. More than one type of centre may be present, for example

mixtures of amino groups may be accommodated, and/or sulphonium and/or phosphonium groups.

In one general preference each cationic centre is an amino group. Particularly useful amino groups include primary and secondary amino groups. The number of cationic centres in the cationic head (1) will preferably be from three to six.

Each cationic centre will in general be separated from any other centre by spacer groups arranged to link the centres in a linear (straight and/or branched) or cyclic fashion. The overall effect may be a cationic head (1) which has a straight and/or branched linear structure, a cyclic structure, or a mixture of straight and/or branched linear and cyclic structures. More than one type of spacer group may be present in a cationic head (1). Where desired a spacer group may form a terminal group on the cationic head (1), acting as a substituent on a cationic centre rather than a group connecting centres together.

Each spacer group will in general be non-ionic and contain at least one carbon atom. Suitable groups include optionally substituted aliphatic, cycloaliphatic, heteroaliphatic, heterocycloaliphatic, aromatic or hetero-aromatic groups.

Particular examples of optionally substituted aliphatic spacer groups include optionally substituted C_{1-10}aliphatic chains such as optionally substituted straight or branched C_{1-6}alkylene, C_{2-6}alkenylene or C_{2-6}alkynylene chains.

Heteroaliphatic spacer groups include the aliphatic chains just described but with each chain additionally containing one, two, three or four heteroatoms or heteroatom-containing groups. Particular heteroatoms or groups include atoms or groups L^2 where L^2 is as defined below for L^1 when L^1 is a linker atom or group. Each L^2 atom or group may interrupt the aliphatic chain, or may be positioned at its terminal carbon atom to connect the chain to the atom or group R^1.

Particular examples of aliphatic spacer groups include optionally substituted $-CH_2-$, $-CH_2CH_2-$, $-CH(CH_3)-$, $-C(CH_3)_2-$, $-(CH_2)_2CH_2-$, $-CH(CH_3)CH_2-$, $-(CH_2)_3CH_2-$, $-CH(CH_3)CH_2CH_2-$, $-CH_2CH(CH_3)CH_2-$, $-C(CH_3)_2CH_2-$, $-(CH_2)_4CH_2-$, $-(CH_2)_5CH_2-$, $-CHCH-$, $-CHCHCH_2-$, $-CH_2CHCH-$, $-CHCHCH_2CH_2-$, $-CH_2CHCHCH_2-$, $-(CH_2)_2CHCH-$, $-CC-$, $-CCCH_2-$, $-CH_2CC-$, $-CCCH_2CH_2-$, $-CH_2CCCH_2-$, or $-(CH_2)_2CC-$ chains. Where appropriate each of said chains may be optionally interrupted by one or two atoms and/or groups L^2 to form an optionally substituted heteroaliphatic spacer group. Particular examples include optionally substituted $-L^2CH_2-$, $-CH_2L^2CH_2-$, $-L^2(CH_2)_2-$, $-CH_2L^2(CH_2)_2-$, $-(CH_2)_2L^2CH_2-$, $-L^2(CH_2)_3-$ and $-(CH_2)_2L^2(CH_2)_2-$ chains. The optional substituents which may be present on aliphatic or heteroaliphatic spacer groups include one, two, three or more substituents selected from halogen atoms, e.g. fluorine, chlorine, bromine or iodine atoms, or hydroxyl, C_{1-6}alkoxy, e.g. methoxy or ethoxy, haloC_{1-6}alkoxy, e.g. halomethoxy or haloethoxy such as difluoromethoxy or trifluoromethoxy, thiol, or C_{1-6}alkylthio e.g. methylthio or ethylthio. Particular examples of substituted spacer groups include those specific chains just described substituted by one, two, or three halogen atoms such as fluorine atoms, for example chains of the type $-CH(CF_3)-$, $-C(CF_3)_2-$ $-CH_2CH(CF_3)-$, $-CH_2C(CF_3)_2-$, $-CH(CF_3)-$ and $-C(CF_3)_2CH_2-$.

Optionally substituted cycloaliphatic spacer groups in the cationic head (1) include optionally substituted C_{3-10} cycloaliphatic groups. Particular examples include optionally substituted C_{3-10}cycloalkylene, e.g. C_{3-7}cycloalkylene, C_{3-10}cycloalkenylene e.g. C_{3-7}cycloalkenylene or C_{3-10}cycloalkynylene e.g. C_{3-7}cycloalkynylene groups.

Particular examples of cycloaliphatic spacer groups include optionally substituted cyclopropylene, cyclobutylene, cyclopentylene, cyclohexylene, cycloheptylene, 2-cyclobuten-1-ylene, 2-cyclopenten-1-ylene and 3-cyclopenten-1-ylene groups.

Optionally substituted heterocycloaliphatic spacer groups include the optionally substituted cycloaliphatic groups just described but with each

group additionally containing one, two, three or four heteroatoms or heteroatom-containing groups L^2 as just defined.

The optional substituents which may be present on the cycloaliphatic or heterocycloaliphatic spacer groups include one, two, three or more substituents selected from halogen atoms C_{1-6}alkyl, e.g. methyl or ethyl, haloC_{1-6}alkyl, e.g. halomethyl or haloethyl such as difluoromethyl or trifluoromethyl, hydroxyl, C_{1-6}alkoxy, e.g. methoxy or ethoxy, haloC_{1-6}alkoxy, e.g. halomethoxy or haloethoxy such as difluoromethoxy or trifluoromethoxy, thiol, or C_{1-6}alkylthio e.g. methylthio or ethylthio groups.

Optionally substituted aromatic spacer groups include for example monocyclic C_{6-12} aromatic groups, such as optionally substituted phenylene.

Optionally substituted heteroaromatic spacer groups, include for example optionally substituted monocyclic C_{1-9} heteroaromatic groups containing for example one, two, three or four heteroatoms selected from oxygen, sulphur or nitrogen atoms. Monocyclic heteroaromatic groups include for example five- or six-membered heteroaromatic groups containing one, two, three or four heteroatoms selected from oxygen, sulphur or nitrogen atoms.

Optional substituents which may be present on the aromatic or heteroaromatic spacer groups include one, two, three or more substituents selected from those just described in relation to cycloaliphatic and heterocycloaliphatic spacer groups.

In one general preference each spacer group in the cationic head (1) is preferably an optionally substituted straight or branched C_{1-6}alkylene chain. Particularly useful chains include $-(CH_2)_2-$, $-(CH_2)_3-$ and $-(CH_2)_4-$ chains.

The hydrophobic backbone (2) in the lipids according to the invention may comprise one or more hydrocarbon chains. Each hydrocarbon may be for example an optionally substituted straight or branched aliphatic or

heteroaliphatic chain containing a minimum of ten up to a maximum of around one hundred chain-linked atoms as described in more detail below. The hydrocarbon may be attached either directly or indirectly through a linker atom or group to the cationic head (1). Particular examples of suitable linker groups are those represented by the group L^1 described below.

At least one heteroatom in a cationic centre in the lipids of the invenion is covalently linked to a hydrocarbon chain of the hydrophobic backbone (2). Where desired any other available heteroatom in the same or any other cationic centre may be additionally linked to the same or other hydrocarbon chains making up the backbone (2). It is generally preferred however to link the backbone (2) and cationic head (1) at one heteroatom in one cationic centre. Thus a preferred class of lipids according to the invention has one or two hydrocarbon chains as just described indirectly linked through a linker atom or group to a heteroatom in a cationic centre in the cationic head (1).

The hydrophilic tail (3) in the lipids according to the invention may in general be one or more acyclic hydrophilic hydrocarbons having little or no overall positive or negative charge and containing a minimum of two up to a maximum of around one hundred atoms or groups capable of being solvated by water. Each hydrophilic hydrocarbon in the hydrophilic tail (3) may be attached directly or indirectly through a linker atom or group to a hydrocarbon chain of the hydrocarbon backbone (2). The attachment point may be anywhere on the hydrocarbon chain provided that it is at least ten atoms along the chain, excluding branches, from the terminal carbon atom connecting the hydrophobic backbone (2) to the cationic head (1). In one general preference the attachment point may be at a terminal carbon atom of a hydrocarbon chain distal to the chain carbon atom attached to the cationic head (1). Particular examples of suitable acyclic hydrophilic hydrocarbons which constitute the hydrophilic tail (3) are described in more detail below.

A particularly useful group of lipids according to the invention may be represented by the formula (1):

$$[R^1]_m\text{-}(L^1)_n\text{-}R^2 \qquad (1)$$

wherein R^1 is a hydrocarbon chain optionally substituted by one or more acyclic hydrophilic hydrocarbons each containing two or more atoms or groups capable of being solvated by water, provided that at least one hydrocarbon chain is substituted by at least one hydrophilic hydrocarbon and each hydrophilic hydrocarbon is attached to the hydrocarbon chain to achieve at least a ten atom spacing along the chain between the hydrophilic hydrocarbon and the group $-(L^1)_n-[R^2$
m is an integer from 1 to 6;
L^1 is a linker atom or group;
n is zero or the integer 1;
R^2 is an optionally substituted aliphatic, cycloaliphatic, heteroaliphatic, heterocycloaliphatic, aromatic or heteroaromatic group containing two or more cationic centres, and provided that each $[R^1]_m-(L^1)_n-$ group is attached to a heteroatom in a cationic centre in R^2;
and the salts, solvates and hydrates thereof.

In the compounds of formula (1), the optionally substituted aliphatic, cycloaliphatic, heteroaliphatic, heterocycloaliphatic, aromatic or heteroaromatic group represented by R^2, may be an optionally substituted C_{1-30} aliphatic, C_{3-10} cycloaliphatic, C_{1-30} heteroaliphatic, C_{3-10} heterocycloaliphatic, C_{6-12} aromatic or C_{1-9} heteroaromatic group, containing two or more cationic centres. Particular examples of such groups include those generally and specifically described above in relation to the spacer groups present in the cationic head (1) with the additional presence of one or more cationic centres as defined herein.

In general in the lipids of the invention when the hydrophobic backbone (2) and cationic head (1) are joined indirectly by a linker atom or group, as represented by L^1 in compounds of formula (1) when n is 1, then the linker atom or group may be any multivalent atom or group. Particular examples of suitable linker atoms or groups include those of formula $-(Alk^1)_r(X^1)_s(Alk^2)_t-$ where X^1 is an -O- or -S- atom or a -C(O)-, -C(O)O-, -C(S)-, -S(O), $-S(O)_2-$ $-N(R^3)-$, [where R^3 is a hydrogen atom, a straight or branched alkyl group such as a methyl or ethyl group or an $-Alk^1X^1-$

chain], -CON(R^3)-, -OC(O)N(R^3)-, -CSN(R^3)-, -N(R^3)CO-, N(R^3)C(O)O-, -N(R^3)CS-, -S(O)N(R^3)-, -S(O)$_2$N(R^3)-, -N(R^3)S(O)-, -N(R^3)S(O)$_2$-, -N(R^3)CON(R^3)-, or -N(R^3)SO$_2$N(R^3)- group [where any of these groups contains two R^3 substituents these may be the same or different]; Alk^1 and Alk^2 which may be the same or different is each an optionally substituted straight or branched C_{1-6}alkylene, C_{2-6}alkenylene or C_{2-6}alkynylene chain optionally interrupted or terminated by one or more, e.g. one, two or three, carbocyclic or heterocarbocyclic groups and/or heteroatoms or heteroatom containing groups X^1 as just defined, and r, s, and t, which may be the same or different, is each zero or the integer 1, provided that when one of r, s or t is zero at least one of the remainder is the integer 1.

Carbocyclic groups which may interrupt the groups Alk^1 and Alk^2 include for example optionally substituted C_{4-8}cycloalkyl, e.g. optionally substituted cyclopentyl or cyclohexyl groups, or optionally substituted C_{4-8}cycloalkenyl, e.g. optionally substituted cyclopentenyl or cyclohexenyl groups. Heterocarbocyclic groups include for example carbocyclic groups of the types just mentioned containing one or more heteroatoms or heteroatom-containing groups X^1 as defined above. Optional substituents which may be present on the chains represented by Alk^1 and Alk^2 and the carbocyclic or heterocarbocyclic groups which can interrupt or terminate them include one, two or three substituents selected from halogen atoms, e.g. fluorine, chlorine, bromine or iodine atoms or C_{1-3}alkyl, e.g. methyl or ethyl, or C_{1-3}alkoxy e.g. methoxy or ethoxy groups.

It will be appreciated that the linker atom or group will be at least divalent in the instance where one hydrocarbon chain in the hydrophobic backbone (2) is attached to it. Where it is desired to attach more than one hydrocarbon chain to the linker the latter will need to be selected with an appropriate valency and this will generally mean that at least one of Alk^1 or Alk^2 will need to be present in a branched form and with the requisite number of X^1 atoms or groups to achieve the desired coupling.

Particular examples of linker groups include groups of formula -X^1Alk^2- where X^1 is as defined above and Alk^2 is an optionally substituted -CH_2-, -$(CH_2)_2$-, -$(CH_2)_3$-, -$(CH_2)_4$-, -$(CH_2)_5$- or -$(CH_2)_6$- chain; groups of formula

$[X^1]_2Alk^1X^1Alk^2$ where Alk^1 is a $-CH_2CH<$ group and X^1 and Alk^2 are as just defined or a group of formula $[X^1]_2Alk^1Alk^2$ where X^1, Alk^1 and Alk^2 are as just defined.

Each hydrocarbon chain in the hydrophobic backbone (2) of the lipids according to the invention and as represented by R^1 in compounds of formula (1) may be a C_{10} up to about a C_{60} hydrocarbon chain, for example a C_{16} to C_{60} hydrocarbon chain such as a C_{18} to C_{48} hydrocarbon chain.

In particular, the chain may be an optionally substituted C_{10-60} aliphatic chain such as an optionally substituted straight or branched C_{10-60}alkylene, C_{10-60}alkenylene or C_{10-60}alkynylene chain. Optional substituents which may be present on such chains include one or more halogen atoms, e.g. fluorine, chlorine, bromine or iodine atoms, or haloC_{1-6}alkyl, e.g. $-CF_3$ groups. Where desired each alkylene, alkenylene or alkynylene chain may be interrupted by one or more oxygen or sulphur atoms or optionally substituted C_{5-7}cycloalkyl, e.g. cyclopentyl or cyclohexyl, C_{5-7}cycloalkenyl, e.g. cyclopentenyl or cyclohexenyl, -C(O)-, -C(S)-, $-C(O)N(R^3)-$, $-C(S)N(R^3)-$, $-N(R^3)C(O)-$, $-N(R^3)C(S)-$, -C(O)O-, -C(O)S-, $-OC(O)N(R^3)-$, -S(O)-, $-S(O_2)-$, $-S(O)N(R^3)-$, $-S(O)_2N(R^3)-$, $-N(R^3)S(O)-$, $-N(R^3)S(O)_2-$, $-N(R^3)C(O)N(R^3)-$, $-N(R^3)C(S)N(R^3)-$, $-N(R^3)S(O)N(R^3)-$ or $-N(R^3)S(O)_2N(R^3)-$ groups. Optional substituents which may be present on cycloalkyl or cycloalkenyl groups of this type include one or more halogen atoms or haloalkyl groups as just described. It will be appreciated that when the hydrocarbon chain in the hydrophobic backbone (2) is an alkenylene or alkynylene chain it may have more than one unsaturated group.

As generally explained above, the hydrophilic tail (3) in the lipids according to the invention may be formed by one or more acyclic hydrophilic hydrocarbons, each attached to a hydrocarbon chain in the hydrophobic backbone (2), for example as generally represented by R^1 in compounds of formula (1). Each hydrophilic hydrocarbon may be an aliphatic or heteroaliphatic group. Particular examples of aliphatic groups include alkyl, alkenyl or alkynyl groups. Each of these groups may be optionally

interrupted by one or more heteroatoms or heteroatom-containing groups, for example of the type described above in relation to the group L^1 to yield heteroaliphatic groups. In general, each hydrophilic hydrocarbon group forming the hydrophilic tail (3) may contain from one carbon atom to around two hundred carbon atoms.

Each hydrophilic hydrocarbon contains two or more atoms or groups capable of being solvated by water. Examples of such groups include oxygen atoms (-O-) or oxygen-containing groups. Oxygen atoms may form part of a heteroaliphatic group as just described. Oxygen-containing groups may be substituents present on the various hydrocarbons just mentioned and include for example hydroxyl, amide and alkoxy groups such as methoxy or ethoxy groups. In general the number of groups capable of being solvated by water in each hydrocarbon will range from two to around two hundred.

Particular examples of suitable hydrophilic hydrocarbons include polyols. Suitable polyols include naturally occurring polyols such as acyclic sugars and derivatives thereof, and synthetic polyols. Particular sugars include acyclic mono- and oligosaccharides. Sugar derivatives include acyclic glycosides in which a non-ionic aliphatic or heteroaliphatic group (for example of the type described herein) is joined to a sugar by a glycosidic linkage. Acyclic monosaccharides include for example open-chain compounds containing three to eight, e.g. five or six, carbon atoms and at least two hydroxyl substituents. Acyclic oligosaccharides include for example at least two open-chain monosaccharides as just defined linked together by a glycosidic linkage. More than one type of open-chain monosaccharide may be present to yield a homo- or heterooligosaccharide.

Alternatively the hydrophilic hydrocarbon may be a polyether, for example a poly(alkylene oxide) and derivatives thereof, such as poly(ethylene oxide), poly(propylene oxide) or methoxy poly(ethylene oxide), a poly(oxyalkylated alcohol) or a poly(alkenylene alcohol) or poly(alkynylene alcohol) such as poly(vinyl alcohol). The hydrocarbons may in general be

straight or branched. Where desired co-polymers of these hydrocarbons may be used.

Each hydrophilic hydrocarbon may be linked directly or indirectly to a hydrocarbon chain in the hydrophobic backbone (2). For indirect linkage a linker atom or group may be employed, for example an atom or group L^3 where L^3 is as defined above as for the linker atom or group L^1. Where the group L^3 is multivalent, for example when it is a branched alkylene chain containing more than one X^1 atom or group, more than one hydrophilic hydrocarbon may be attached to it.

A particularly useful group of compounds according to the invention has the formula (1a):

$$[R^7]_p\text{-}(L^3)_q\text{-}[R^6]_m\text{-}(L^1)_n\text{-}R^2 \qquad (1a)$$

wherein R^2, L^1, m and n are as defined for formula (1);
R^6 is a hydrocarbon chain;
L^3 is a linker atom or group;
R^7 is a hydrophilic hydrocarbon containing two or more atoms or groups capable of being solvated by water;
q is zero or an integer from one to six;
p is an integer from one to six;
and the salts, solvates and hydrates thereof, provided that each R^7 or L^3 group, when present, is attached to a group R^6 to achieve at least a ten atom spacing along R^6 between R^7 or L^3 and the group $-(L^1)_n-R^2$.

In the compounds of formula (1a) the hydrocarbon chain represented by R^6 may be a C_{10} up to about a C_{60} hydrocarbon chain as generally and more particularly described above in relation to the group R^1. The hydrophilic hydrocarbon R^7 may similarly be a hydrophilic hydrocarbon as described previously in relation to the group R^1. The group L^3 may be a linker atom or group as just defined.

The cationic head (1) in the lipids according to the invention will preferably be a group R^2 as described above in relation to the compounds of

formulae (1) and (1a). In groups of this type, R^2 is preferably a group $-WSp^1[WSp^2]_bWSp^3$ or $-WSp^1[WSp^2]_bWH$ in which Sp^1, Sp^2 and Sp^3, which may be the same or different, is each a spacer group as defined above, W is a cationic centre as defined herein and b is zero or an integer from one to six.

In particular groups of this type, the cationic centre W is preferably a -NH- group. Sp^1, Sp^2 and Sp^3, which may be the same or different, is each preferably an optionally substituted C_{1-6}alkylene chain. b is preferably an integer from two to four.

Particularly useful cationic heads (1) in compounds of the invention include those of formula $-NH[Sp^1NHSp^2]NH_2$, $-NH[Sp^1NHSp^2NHSp^2]NH_2$ or $-NH[Sp^1NHSp^2NHSp^2]NHCH_3$ where each Sp^1 and Sp^2 group is the same or different and is an optionally substituted C_{1-6}alkylene chain, particularly wherein Sp^1 is $-CH_2-$ and each Sp^2 is $-(CH_2)_3-$ or $-(CH_2)_4-$.

In general in the lipids according to the invention the hydrophobic backbone (2) preferably comprises two or, especially one hydrocarbon chain as defined herein. Thus m in formulae (1) and (1a) is preferably an integer 2 or, especially, an integer 1. Each hydrocarbon chain, for example as represented by R^1 and R^6 in formulae (1) and (1a) respectively, is preferably linear and in particular is a linear, optionally substituted C_{16-38}alkylene chain. Optionally substituted C_{18-24}alkylene chains are particularly useful.

In general each hydrocarbon chain in the hydrophobic backbone (2) is preferably linked indirectly to the cationic head (1) through a linker atom or group. The linker atom or group may be for example an atom or group L^1 as defined herein and thus in the compounds of formulae (1) and (1a) for example n is preferably the integer 1.

Preferred linkers include those of formula $-X^1Alk^2-$ or $-[X^1]_2Alk^1X^1Alk^2-$ where X^1, Alk^1 and Alk^2 are as defined previously. Particularly useful linkers of these types are those wherein Alk^2 is a $-(CH_2)_4-$, $-(CH_2)_5-$ or,

especially, $-(CH_2)_6-$ chain. X^1 in these linkers is preferably a -CONH- group. Alk^1 when present is preferably a $-CH_2-CH<$ chain.

In another general preference each hydrocarbon chain in the hydrocarbon backbone (2) has two, or especially one, acyclic hydrophilic hydrocarbon attached to it. Each hydrophilic hydrocarbon is preferably attached to the terminal carbon atom of the hydrocarbon chain distal to the chain carbon atom attached to the cationic head (1). Preferably the hydrophilic hydrocarbon and hydrocarbon chain are indirectly linked through a linker atom or group. Thus in one particular preference in compounds of formula (1a) q is the integer 1 and p is the integer 1 or 2.

In compounds of this type and in general the group L^3 may preferably be an atom or group $-X^1-$, $-X^1Alk^1X-$ or $[X^1Alk^1]_2X^1Alk^2X^1-$. Particularly useful L^3 groups include -NHCO-, -CONH-, $-CONH(CH_2)_2NHCO-$, or $-[CONH(CH_2)_2-]_2NCO(CH_2)_2CONH-$ groups.

In general, the hydrophilic hydrocarbon, for example as represented by R^7 in formula (1a) is preferably a synthetic polyol, a naturally occurring polyol such as an acyclic mono- or disaccharide, or a poly(alkylene oxide) as defined herein. In particular R^7 may be a poly(alkylene oxide) or a derivative thereof, especially a poly(ethylene oxide).

Particularly useful lipids according to the invention are those described in the Examples hereinafter.

The lipids according to the invention may generally be prepared by coupling appropriately functionalised cationic heads (1), hydrophobic hydrocarbons (2) and hydrophilic hydrocarbons (3) in a predetermined order. Standard chemical coupling techniques may be employed utilising starting materials containing one or more reactive functional groups such as acids, thioacids, anhydrides, acid halides, esters, imides, aldehydes, ketones, alcoholc and amines. Illustrative reactions are described in detail in the Examples hereinafter for the preparation of a number of lipids according to the invention and these may be readily adapted using different starting materials to provide other compounds of the invention.

Thus in one general approach a homo- or heterobifunctional hydrocarbon chain may first be coupled to a hydrophilic hydrocarbon or cationic head and the resulting product coupled as necessary to the remaining component to provide the lipid of the invention.

The homo- or heterobifunctional hydrocarbon chain may be any hydrocarbon chain described herein containing two different reactive functional groups of the types just described. Particularly useful groups include acids and thioacids and reactive derivatives thereof, and amines. These can be used to participate in acylation or thioacylation reactions to couple the hydrocarbon chain to an amine or acid as appropriate in any suitable hydrophilic hydrocarbon and/or cationic head.

Acylation or thioacylation may be achieved using standard conditions for reactions of this type. Thus, for example the reaction may be carried out in a solvent, for example an inert organic solvent such as an amide, e.g. a substituted amide such as dimethylformamide, an ether, e.g. a cyclic ether such as tetrahydrofuran, or a halogenated hydrocarbon, such as dichloromethane, at a temperature from around ambient temperature to the reflux temperature, optionally in the presence of a base such as an amine, e.g. triethylamine, or a cyclic amine, such as 1,8-diazabicyclo[5.4.0]undec7--ene, pyridine, dimethylaminopyridine, or N-methylmorpholine.

Where an acid is used the acylation may additionally be performed in the presence of a condensing agent, for example a diimide such as 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide or N,N'-dicyclohexylcarbodiimide, advantageously in the presence of a catalyst such as a N-hydroxy compound e.g. a N-hydroxytriazole such as 1-hydroxybenzotriazole or a N-hydroxyimide such as N-hydroxysuccinimide. Alternatively, the acid may be reacted with a chloroformate, for example ethylchloroformate, prior to reaction with the amine.

In the heterobifunctional hydrocarbon chain one of the reactive functional groups may need to be in a protected form prior to any coupling reaction to avoid its unwanted participation in the reaction. Similarly other

functional groups when present in the hydrocarbon chain, or the intermediates used to generate the hydrophilic hydrocarbon and/or the cationic head may need to be in a protected form before these reagents can be used. Conventional protecting groups may be used in accordance with standard practice [see, for example, Green, T. W. in "Protective Groups in Organic Synthesis", John Wiley & Sons, 1991 and the Examples hereinafter].

Suitable heterobifunctional hydrocarbon chains are either known, readily available materials or may be obtained by synthesis using conventional techniques for example as described in the Examples hereinafter. Thus generally a heterobifunctional hydrocarbon chain of any desired length may be synthesised in one or more reactions using appropriately functionalised shorter chains. Thus in one example a shorter chain aldehyde may be reacted with a shorter chain phosphonium salt to yield a longer chain olefin of the desired length. In this particular example the reaction may be carried out in the presence of a base, for example an organometallic base such as an organolithium compound, a hydride such as sodium or potassium hydride or an alkoxide such as a sodium alkoxide e.g. sodium methoxide. The reaction may be performed in a suitable solvent, for example a polar aprotic solvent such as an alkyl sulphoxide, e.g. dimethylsulphoxide at a low temperature, for example around 0C°. The starting aldehyde and phosphonium salt may be obtained from known starting alcohols and halides respectively using conventional procedures. Where desired, the olefin obtained above may be hydrogenated using hydrogen and a catalyst, for example Pearlman's catalyst, to yield the corresponding saturated hydrocarbon chain.

Where it is desired to obtain hydrocarbon chains containing one or more heteroatoms or heteroatom-containing groups these may be synthesised from smaller chains containing functional groups which can be chemically coupled, for example by acylation or thioacylation as generally described above.

Suitable functionalised hydrophilic hydrocarbons or cationic heads for coupling to the heterobifunctional hydrocarbon chain are either readily

available or may be synthesised from known materials by conventional methods for example as described in the Examples hereinafter.

The advantageous properties of the lipids according to the invention may be demonstrated using the small scale tests described hereinafter in the Examples. In these the lipids can be shown to efficiently compact any bioactive substance, and to self-assemble with the substance in aqueous solution to yield stable complexes which remain associated over wide pH and ionic strength ranges and which will efficiently deliver the substance to eucaryotic cells.

The lipids can thus be expected to be of use for the delivery of bioactive substances to cells, particularly eucaryotic cells, *in vitro* and especially *in vivo*. Particular general uses to which the lipids may be put thus include for the delivery of bioactive substances to cells in culture, and in human medicine for the delivery of therapeutic or diagnostic agents, or agents which can generate a host immune response for vaccine or other immuno-modulatory purposes. The lipids are particularly well suited for delivering bioactive polyanions, especially nucleic acids, and are of particular use to modify a host's genotype or its expression.

Thus, in another aspect of the invention we provide a lipid complex characterised in that it comprises a bipolar lipid comprising a cationic head (1) a hydrophobic backbone (2) and a hydrophilic tail (3) in which:

(A) the cationic head comprises two or more cationic centres, each centre containig at least one heteroatom and being covalently linked to one or more other centres, by one or more carbon containing spacer groups;

(B) the hydrophobic backbone comprises one or more hydrocarbon chains; and

(C) the hydrophilic tail comprises one or more acyclic hydrophilic hydrocarbons each containing two or more atoms or groups capable of being solvated by water;

each of said components (1) to (3) being covalently linked head (1) to backbone (2) to tail (3) and arranged such that at least one hydrocarbon chain in the hydrophobic backbone (2) is covalently linked to a heteroatom

in a cationic head (1) and each hydrophilic hydrocarbon in the hydrophilic tail (3) is covalently linked to a chain in the backbone (2) to achieve at least a carbon atom spacing along the chain between the tail (3) and the head (1),
in association with one or more bioactive substances.

In the complexes according to the invention, each bioactive substance may be for example a pharmacologically active agent, a diagnostic agent or any agent able to modify the genotype and/or phenotype of a cell.

Particular examples of such substances include bioactive proteins, peptides, polysaccharides, nucleic acids including synthetic polynucleotides, oligonucleotides and derivatives thereof, lipids, glycolipids, lipoproteins, lipopolysaccharides and viral, bacterial, protozoal, cellular or tissue fractions.

Where desired the complexes according to the invention may contain two or more different bipolar lipids of the invention. Especially useful mixtures include those containing two or more bipolar lipids of the invention which differ from each other in the nature of the hydrophilic tail present in each. The proportion of each lipid in complexes of this type may be manipulated to obtain complexes with different physio-chemical properties, for example overall surface charge and/or particle size, tailored to meet the intended use of the complex. Thus for example in one advantageous lipid complex containing two or more bipolar lipids, one of the lipids has a hydrophilic tail formed by a poly(alkyene oxide) or a derivative thereof as defined herein, while each of the others has a hydrophilic tail formed by a synthetic or naturally occurring polyol as described previously. The proportion of the first poly(alkylene oxide)-containing lipid may be varied in such complexes so that the mole ratio of first lipid to second and other lipids is from 1:10000 to 1:1, advantageously from around 1:1000 to around 1:20, especially around 1:10. Complexes of these types, particularly where the poly(alkylene oxide) is poly(ethylene oxide), may be obtained which advantageously have zero surface charge and do not aggregate when left in solution and which additionally are able to compact a bioactive

substance to give small particles of 150nm and below, particularly 100nm and below, especially around 80-85nm.

The lipids according to the invention are particularly suited for delivering polyanions to cells and preferred lipid complexes of the invention thus include lipid-polyanion complexes in which the polyanion may be any of the above-mentioned bioactive substances possessing a net negative charge. Particular polyanions include nucleic acids, for example single or double stranded, circular or supercoiled DNA or RNA and derivatives thereof. Where desired the DNA may be part of a structure such as a plasmid.

The lipid complexes will in general comprise a lipid according to the invention and a bioactive substance in a weight ratio of around 0.1:1 to around 100:1, for example around 1:1 to around 50:1. The complexes may be formed as liquids, by initially mixing one or more bipolar lipids according to the invention, and a bioactive substance together advantageously in an aqueous solvent using conventional procedures. Where desired the solvent may be removed, for example by lyophilisation, to obtain a solid lipid complex.

Where desired the lipid complexes may be targeted lipid complexes in which the lipid complex is assembled with one or more, particularly one targeting molecules, and the invention extends to such assemblies. The targeting molecule may be for example a member of a complementary binding pair, the other member of the pair being present in a mammalian, e.g. human, or other animal, insect, microbial or plant host either attached to a cell membrane or other cell surface or in soluble form and present intracellularly and/or extracellularly. Thus in general the targeting molecule may be a peptide, including a glycopeptide, a polypeptide, protein, including a glycoprotein or phosphoprotein, a carbohydrate, glycolipid, phospholipid, oligonucleotide, polynucleotide or other organic molecule, e.g. a vitamin, which can specifically bind to a receptor, ligand, antigen or other naturally occurring or synthetic organic molecule.

The binding affinity of the targeting molecule for the other member of the complementary pair will be at least 10^{-5}M, preferably 10^{-7}M and greater, e.g. around 10^{-8}M to around 10^{-12}M. Preferably the targeting molecule will be selective for the other member of the pair and it will not cross-react although absolute specificity is not essential. Advantageously the interaction of the targeting molecule with its ligand leads to delivery of the lipid complex to the cell interior.

Antibodies and antigen-binding fragments and derivatives thereof such as Fab, Fab' and single chain F_V fragments form one particular class of suitable targeting molecules. Usefully the antibody is a whole antibody, particularly an IgG antibody, or a Fab, Fab' or single chain F_V thereof. Advantageously the antibody, fragment or derivative is an internalising antibody, fragment or derivative.

Other examples of suitable targeting molecules include antibody mimetic molecules produced by combinatorial or other synthetic means; interferons, for example interferons α, β and γ; tumour necrosis factors α and β; interleukins, for example interleukins 1 to 15; chemokines, for example MIP-1α, MIP-1β and RANTES; growth factors, for example PDGF, VEGF, EGF, TGFα, TBFβ, GM-CSF, G-CSF, M-CSF, FGF, IGF, bombesins, thrombopoietin, erythropoietin, oncostatin and endothelin 1; peptide hormones, for example LH, FSH, TRH, TSH, ACTH, CRH, PRH, MRH, MSH, glucagon and prolactin; transferrin; lactoferrin; angiotensin; histamine; insulin; lectins; tissue inhibitor of metalloproteinases, for example TIMP-1, TIMP-2 and TIMP-3; apolipoproteins, for example apolipoprotein E; kinins; and vitamins, for example folic acid and vitamin B12. Fragments and other synthetic analogues of these molecules may be used, where these retain or have the appropriate selective binding action. It will be appreciated that the above list is not exhaustive and may be extended to include other naturally occurring binding molecules, including for example the complementary binding partner, or a binding fragment thereof, of each of those mentioned, for example the PDGF receptor, the VEGF receptor and so on.

Similarly, adhesion moelcules and their binding partners or binding fragments thereof may be used in the invention as targeting molecules. Particular examples include VLA-4, VMAC-1, fibronectin, LFA-1, MAC-1,ICAM-1, ICAM-2, Lewis X, GMP-140, ELAM-1, S-Lewis X, fibrinogen, GPIIb/IIIa, CD28, B7, CD40, CD402L, CD4, laminin, VLA-1, VLA-2, VLA-3 and VLA-6.

Other examples of suitable targeting molecules include monosaccharides and oligosaccharides such as galactose, lactose and mannose.

The targeting molecule may be present in the targeted lipid complex either non-covalently associated with or covalently linked to a bipolar lipid of the invention or to any other lipid or other component which may be present as part of a formulation as described below. Covalently linked targeted lipid complexes may be obtained by coupling together a targeting molecule and a bipolar or other lipid or other component using standard chemical coupling techniques and appropriate functional groups present in the starting materials, for example as described above for the preparation of the bipolar lipids of the invention.

The lipid complexes according to the invention including targeted lipid complexes, may be formulated with other materials such as one or more other lipids or other pharmaceutically acceptable carriers, excipients or diluents and the invention extends to such compositions. In this aspect of the invention the "other" lipid may be for example selected from any known neutral and/or cationic lipid, for example selected from those described herein in the introduction to the invention (see page 2) and also especially including DOPE and other cholesterol derivatives such as cholesterol hemisuccinate. Particularly useful formulations of this type are those wherein the bipolar lipid of the invention has a poly(alkylene oxide) tail, especially a poly(ethylene oxide) tail.

Particular compositions include liposome formulations, prepared using conventional liposome technology. Otherwise, the compositions may take any other form suitable for oral, buccal, parenteral, nasal, topical or rectal

administration, or a form suitable for administration by inhalation or insufflation.

For oral administration, the compositions may take the form of, for example, tablets, lozenges or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g. pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g. lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g. magnesium stearate, talc or silica); disintegrants (e.g. potato starch or sodium glycollate); or wetting agents (e.g. sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents, emulsifying agents, non-aqueous vehicles and preservatives. The preparations may also contain buffer salts, flavouring, colouring and sweetening agents as appropriate.

Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

The complexes of the invention may be formulated for parenteral administration by injection, including bolus injection or infusion or particle mediated injection. Formulations for injection may be presented in unit dosage form, e.g. in glass ampoule or multi dose containers, e.g. glass vials or a device containing a compressed gas such as helium for particle mediated administration. The compositions for bolus injection or infusion may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilising, preserving and/or dispersing agents. Alternatively, the complex may be in powder form for constitution with a

suitable vehicle, e.g. sterile pyrogen-free water, before use. For particle mediated administration the complex may be coated on particles such as microscopic gold particles.

In addition to the formulations described above, the complexes may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation or by intramuscular injection.

For nasal administration or administration by inhalation, the complexes may be conveniently delivered in the form of an aerosol spray presentation for pressurised packs or a nebuliser, with the use of suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas or mixture of gases.

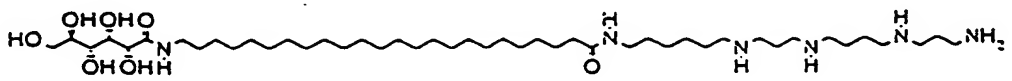
The complexes may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack or dispensing device may be accompanied by instructions for administration.

The quantity of lipid complex required for any particular application will to a large extent depend on the nature of the bioactive substance being delivered. Another important factor will include whether the lipid complex is intended for *in vitro* or *in vivo* use. If the latter the route of administration and particular formulation chosen as well as factors such as the age and condition of the subject will govern the quantity of lipid complex used. In general however up to around 50 mg of lipid complex can be used for every kilogram of body weight.

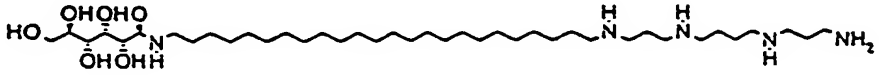
The following Examples illustrate the inventions. In the Examples, the following abbreviations are used: THF - tetrahydrofuran;
Bn - benzyl; DMSO- dimethylsulphoxide; DMF - dimethylformamide.
LDA - lithium diisopropylamide; Ac - acetyl; MeOH - methanol;
DCM - dichloromethane; BOC - t-butyloxycarbonyl;

The compounds of the invention prepared in these Examples are shown below and numbered (14), (19) and (24);

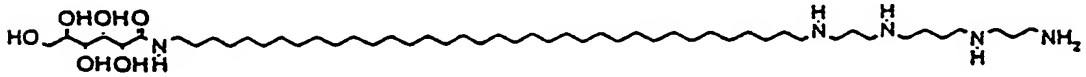
(14) *N*-(aminopropylaminobutylaminopropylaminohexyl)-24-(glucuronylamino) tetracosanamide



(19) *N*-Aminopropylaminobutylaminopropyl-*N'*-glucuronyl-1,24-diaminotetracosane



(24) *N*-aminopropylaminobutylaminopropyl-*N'*-glucuronyl-1,36-diaminohexatriacontane



(1) 12-Aminododecanol hydrochloride

$NH_2(CH_2)_{11}CH_2OH.HCl$

12-Aminododecanoic acid (21.52 g, 100 mmol) was suspended in THF (100 cm^3) and borane-THF complex (500 mmol, 1M solution) added. The reaction was left overnight and carefully quenched with methanol before removal of the solvent. The residues were suspended in 1M HCl (500 cm^3) and heated at 40°C for 1hr and left overnight. The white solid was filtered off, washed with cold 1M HCl, and the product recrystallised from 1M HCl. Yield 18.70 g (79%). Mp 120°C softens, 169°C liquid.

$C_{12}H_{28}N_1O_1Cl.1/5\ H_2O$ requires C: 59.70%, H: 11.86%, N: 5.80%. Found: C: 59.65%, H: 11.82%, N: 5.76%. $C_{12}H_{27}N_1O_1$ requires 201. Found ES+: MH^+ 202.1 (100%). $\delta_H(CD_3CO_2D)$ 3.64 (2H, t, CH_2O), 3.06 (2H, t, NCH_2), 1.73 (2H, m, CH_2CH_2O), 1.57 (2H, m, NCH_2CH_2), 1.2-1.5 (16H, m, CH_2).

(2) 12-(Dibenzylamino)dodecanol

$Bn_2N(CH_2)_{11}CH_2OH$

To **1** (15 g, 63.2 mmol) suspended in a mixture of dichloromethane (150 cm^3) and saturated sodium carbonate in water (150 cm^3) was slowly added benzyl bromide (33.7 g, 23.5 cm^3, 189.6 mmol). The suspension cleared and reaction was complete after 4hr. Aqueous ammonia (0.880,30 cm^3) was added and the reaction left overnight. The organic layer was dried (magnesium sulphate) and evaporated to dryness. The residues were dissolved in refluxing hexane and crystallised at -20°C to yield (18.03 g, 75%).

Mp 45°C. $C_{26}H_{39}N_1O_1$ requires C: 81.84%, H: 10.30%, N: 3.67%. Found: C: 81.64%, H: 10.24%, N: 3.54%. $C_{26}H_{39}N_1O_1$ requires 381. Found ES+: MH^+ 382 (100%). δ_H ($CDCl_3$) 7.1-7.6 (10 H, m, Ar), 3.64 (2H, t, CH_2O), 3.56 (4H, s, $ArCH_2$), 2.41 (2H, t, NCH_2), 1.1-1.8 (22H, dm, CH_2).

(3) 12-(Dibenzylamino)dodecanal

$Bn_2N(CH_2)_{11}CHO$

To a solution of anhydrous dimethylsulphoxide (30 mmol, 2.13 cm^3) in dichloromethane (200 cm^3) at -78°C was added carefully oxalyl chloride (2.6 cm^3, 30 mmol) in dichloromethane (60 cm^3). After 15mins 2 (10 g, 26 mmol) was added in dichloromethane (60 cm^3) and the reaction stirred for 20 mins at -78°C. Triethylamine (28 cm^3) was added dropwise to the cold reaction. A precipitate

formed and after 15mins the reaction was allowed to reach room temperature. Water (100 cm^3) was added to the reaction that was extracted with dichloromethane. The organic layers were washed with water, dried (magnesium sulphate) and evaporated to dryness. The residue was chromatographed (SiO_2, hexane-10% ethyl acetate in hexane) to give the product as an oil (7.97 g, 80%). This compound is unstable and should be used on the day of preparation.

I.R. 1725 cm^{-1} (COH). $C_{26}H_{37}NO$ requires 379.29. Found ES+: MH^+ 380.29. δ_H ($CDCl_3$) 1.32 (14H, br, $(CH_2)_7(CH_2)_2N$), 1.61 (4H, 2xp, CH_2CH_2N, CH_2CH_2CO), 2.43, 2.44 (4H, 2xt, CH_2N, CH_2CO), 3.60 (4H, s, CH_2Ph), 7.2-7.5 (10H, m, Ph), 9.78 (1H, t, COH). δ_C ($CDCl_3$) 22.0, 26.9, 27.1, 29.0, 29.3, 29.4, 29.5 (9C, $(CH_2)_9CH_2N$), 43.8 (1C, CH_2COH), 53.3 (1C, CH_2N), 58.2 (2C, CH_2Ph), 126.6 (2C, $CH(CH)_2C$), 128.0 (4C, CHC), 128.6 (4C, CHCHC), 140.0 (2C, CCH_2N), 202.3 (1C, COH).

(4) 11-(Carboxyundecyl)triphenylphosphonium bromide

$$Ph_3\overset{+}{P}\text{-}(CH_2)_{11}CO_2H\,.\overset{-}{Br}$$

To 12-bromododecanoic acid (3.000 g, 10.7 mmol) suspended in acetonitrile (12 cm^3) was slowly added triphenylphosphine (2.818 g, 10.7 mmol). The reaction was heated at 100°C (no condenser) with argon blowing over the flask until the reaction was a fusion, then maintained at 100°C (with condenser) for 24hrs. The warm residues were dissolved in acetonitrile (18 cm^3) and added dropwise to rapidly stirred cold (dry ice) diethyl ether. The white precipitate formed was then filtered off and the phosphonium salt dried (5.353 g, 92%).

Mp 110-112°C. $C_{30}H_{38}O_2PBr$ requires C: 66.54%, H: 7.07%. Found: C: 66.42%, H: 7.10%. δ_P ($CDCl_3$) 24.3 (s). δ_H ($CDCl_3$) 1.05-1.30 (12 H, br, $(CH_2)_6(CH_2)_2CO_2H$), 1.53 (6H, br, $(CH_2)_2CH_2P$, $CH_2CH_2CO_2H$), 2.28 (2H, t, CH_2CO_2), 3.55 (2H, br, CH_2P), 7.6-7.8 (15H, m, Ph). δ_C ($CDCl_3$) 22.1, 22.3, 22.8, 24.5, 28.8, 28.9, 30.0, 30.2 (10C, $(CH_2)_{10}CO_2H$), 34.2 (1 C, CH_2P), 117.3, 118.7 (3 C, CP), 130.3, 130.5 (6C, CHCHCP), 133.3, 133.5 (6C, CHCP), 134.9 (3C, $CH(CH)_2CP$), 177.4 (1C, CO_2H).

(5) 24-(Dibenzylamino)-12-tetracosenoic acid

$$Bn_2N\text{-}(CH_2)_{11}\text{-}CH{=}CH\text{-}(CH_2)_{10}\text{-}CO_2H$$

4 (13.52 g, 25 mmol) was dissolved in dry DMSO (40 cm^3) under argon at ~0°C (no DMSO solidification). 2.2 equivalents of 2.0M LDA (25 cm^3) were added, the solution turning orange. The reaction was left at 0°C for 30 min, and to the now dark orange solution was added a solution of 3 (7.97 g, 21 mmol) in dry THF (30 cm^3). The solution was maintained at 0°C for 4 hours then added to 2M HCl (50 cm^3). The aqueous layer was extracted with dichloromethane, the fractions combined, dried ($MgSO_4$) and the solvent removed to yield the crude material as a pale yellow gum. Silica column chromatography (30-100% ethyl acetate in hexane) yielded the desired product (6.20 g, 53%), as a pale yellow gum.

$C_{38}H_{59}NO_2$ requires 561.46. Found ES+: MH^+ 562.53, ES-: $(M\text{-}H^+)^-$ 560.55. $\delta_H(CDCl_3)$ 1.26 (30H, br, $(CH_2)_8CH_2CH{=}CHCH_2(CH_2)_7$), 1.42-1.72 (4H, m, $CH_2CH_2CO_2H$, CH_2CH_2N), 2.02 (4 H, dxt, $CH_2CH{=}CHCH_2$), 2.34 (2H, t, CH_2CO_2H), 2.46 (2H, t, CH_2N), 3.65 (4H, s, CH_2Ph), 5.36 (2H, t, CH=CH), 7.2-7.4 (10H, m, Ph). δ_C ($CDCl_3$) 25.0, 26.4, 27.2, 29.3, 29.6 (19C, $(CH_2)_{10}CH{=}CH(CH_2)_9$), 34.5 (1C, CH_2CO_2H), 52.9 (1C, CH_2N), 57.7 (2C, CH_2Ph), 127.0 (2C, $CH(CH)_2C$, 128.2 (4C, CHC), 129.1 (4C, CHCHC), 129.9 (2C, CH=CH), 138.6 (2C, CCH_2N), 179.2 (1C, CO_2H).

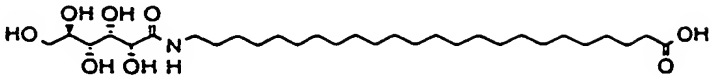
(6) 24-Aminotetracosanoic acid

$$NH_2(CH_2)_{23}CO_2H$$

5 (6.2 g) under an atmosphere of hydrogen was heated at 60°C overnight in glacial acetic acid using Pearlman's catalyst (10% w/w). The reaction was filtered through glass fibre and evaporated to dryness. The product was crystallised from acetic acid / ether (4.2 g, 100%). The product was subjected to high vacuum to remove traces of acetic acid.

Mp 151-155°C. $C_{24}H_{49}NO_2.0.75\ CH_3CO_2H$ requires C: 71.44%, H: 12.23%, N: 3.27%. Found: C: 71.43%, H: 12.15%, N: 3.26%. $C_{24}H_{49}NO_2$ requires 383.38. Found ES+: MH^+ 384.29. δ_H (CD_3OD + TFA) 1.32 (38H, br, $(CH_2)_{19}(CH_2)_2\ NH_2$), 1.65 (4H, br, $CH_2CH_2NH_2$, $CH_2CH_2CO_2H$), 2.33 (2H, t, CH_2CO_2H), 2.74 (2H, m, CH_2NH_2). δ_C (CD_3OD + TFA) partial 33.8 (1C, CH_2CO_2H), 35.3 (1C, CH_2NH_2).

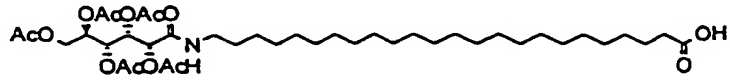
(7) 24-(Glucuronylamino)tetracosanoic acid



A suspension of 6 (792 mg, 2.064 mmol), δ-gluconolactone (1.839 g, 10.32 mmol) and 1,8-diazabicyclo(5.4.0.)undecene (DBU) (4.2 g, 30.9 mmol) in dry methanol (90 cm^3) were heated at 60°C for approximately 10 minutes until all solids had dissolved. The solution was left at room temperature overnight, then the solvent removed. The residues were taken up into water (5 cm^3) and acidified to pH 1 with 1M HCl to precipitate out the desired compound. This was filtered off and dried to yield a white solid (765 mg, 66%). Silica tlc R_f 0.35, ninhydrin negative (1:1:1 methanol:acetic acid:dichloromethane).

I.R. 1581 cm^{-1} (CO_2^-), 1639 cm^{-1} (CONH). δ_H (DMSO) 1.32 (42 H, br, $(CH_2)_{21}CH_2CO_2H$), 2.27 (2 H, t, CH_2CO_2H), 3.15 (2 H, m, CH_2N), 3.3-3.8 (4 H, m, CHOH), 4.0-4.1 (2 H, m, CH_2O).

(8) 24-(Peracetylglucuronylamino)tetracosanoic acid

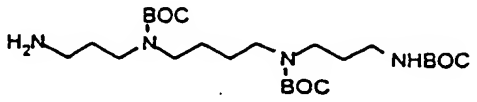


To 7 (765 mg, 1.362 mmol) dissolved in dry pyridine (20 cm^3) was added acetic anhydride (20 cm^3). The solution was stirred under argon overnight and water (50 cm^3) added slowly. The solution was extracted with dichloromethane and the dichloromethane then washed with HCl pH 3 (2 x 20 cm^3) and water (5 x 30 cm^3). The organics were dried ($MgSO_4$) and the solvent removed to yield the penta-acetylated product as a white solid (940 mg, 89%). Alumina tlc R_f 0.15 (15% methanol in dichloromethane).

$C_{40}H_{69}NO_{13}$ requires 771.48. Found ES+: MH^+ 772.07, MNa^+ 794.25. ES-: $(M-H^+)^-$ 770.65. δ_H ($CDCl_3$) 1.26 (38 H, br, $(CH_2)_{19}(CH_2)_2CO_2H$), 1.64 (4 H, m, $CH_2CH_2CO_2H$, CH_2CH_2NH), 2.07, 2.11, 2.13, 2.21 (15 H, s, CH_3CO), 2.35 (2 H, t, CH_2CO_2H), 3.24 (2 H, m, CH_2NH), 4.30 (2 H, 2 x dxd, CH_2OAc), 5.05 (1 H, q, CH(OAc)CH_2OAc), 5.32 (1 H, d, CH(OAc)CONH), 5.46 (1 H, t, CH(OAc)CH(OAc) CH_2OAc), 5.70 (1 H, t, CH(OAc)CH(OAc)CONH), 6.42 (1 H, t, NH). δ_C ($CDCl_3$) 20.4, 24.5, 26.6, 28.8-29.5 (26 C, $(CH_2)_{21}CH_2CO_2H$, CH_3CO), 33.8 (1 C, CH_2CO_2H), 39.3 (1 C, CH_2NH), 61.3 (1 C, CH_2OAc), 68.5, 68.9, 69.1, 71.5 (4 C,

CHOAc), 165.8 (1 C, CONH), 160.0, 169.5, 169.7, 170.4 (5 C, CH_3CO), 178.6 (1 C, CO_2H).

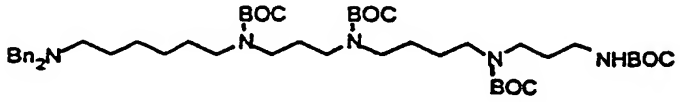
(9) *N,N'-bis*(*t*-Butyloxycarbonyl)-*N*-*t*-butyloxycarbonylaminopropyl-*N'*-aminopropyl-1,4-diaminobutane



To spermine (3.704 g, 18.31 mmol) dissolved in methanol (200 cm^3) at -78°C was added ethyl trifluoroacetate (2.601 g, 18.31 mmol) over 30 minutes. The solution was stirred for a further 30 minutes at -78°C then warmed to 0°C over 1 hour. tert-butyl dicarbonate (15.981 g, 73.23 mmol) was then added as a solid, the reaction allowed to warm to ambient temperature over 1 hour, and a further 2 hours later conc. ammonium hydroxide (approx. 40 cm^3) added until a pH > 11 was achieved. The reaction was stirred overnight at ambient temperature, the solvents removed and the residues purified by silica chromatography (70:10:1 DCM:MeOH:NH_4OH) to yield the product 4.240 g, 46% as a pale yellow oil.

$C_{25}H_{50}N_4O_6$ requires 502.4. Found ES+: MH^+ 503.4. δ_H ($CDCl_3$) 1.44 (31H, m, $C(CH_3)_3$, $NCH_2(CH_2)_2CH_2N$), 1.64 (4H, m, $NCH_2CH_2CH_2N$), 3.14 (2H, t, CH_2NH_2), 3.0-3.4 (10H, m, CH_2N).

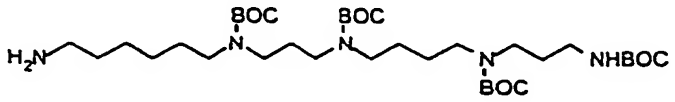
(10) *N*-*t*-Butyloxycarbonyl-*N*-[*t*-butyloxycarbonylaminopropyl(*t*-butyloxycarbonyl)aminobutyl(*t*-butyloxycarbonyl)aminopropyl-*N',N'*-dibenzyl-1,6-diaminohexane



9 (535 mg, 1.064 mmol), 6-(dibenzylamino) hexanal (286 mg, 0.968 mmol) (synthesised as for 3 from 6-aminohexanol) and Na_2SO_4 were stirred overnight in anhydrous methanol (50 cm^3) at ambient temperature under argon. To the filtered reaction was added sodium triacetoxy borohydride (410 mg, 1.94 mmol) and the reaction left for 3 hours at which point water (0.5 cm^3) was added and the reaction left overnight to quench excess reducing agent. To the reaction were added tert-butyl dicarbonate (633 mg, 2.903 mmol) and triethylamine (490 mg, 4.838 mmol) and the reaction left for 3 hours. The solvent was removed and the residues purified by silica column chromatography eluting with 25-50% ethyl acetate in hexane to yield the desired compound 311 mg, 36% as a colourless gum.

$C_{50}H_{82}N_5O_8$ requires 881.6. Found ES+: MH^+ 882.5. δ_H ($CDCl_3$) 1.1-1.8 (52H, m, $(CH_3)_3C$, $Bn_2NCH2(CH_2)_4$, CH_2CH_2N), 2.39 (2H, t, Bn_2NCH_2), 3.0-3.4 (14H, br, CH_2N), 3.54 (4H, s, CH_2Ph), 7.2-7.4 (10H, m, Ph).

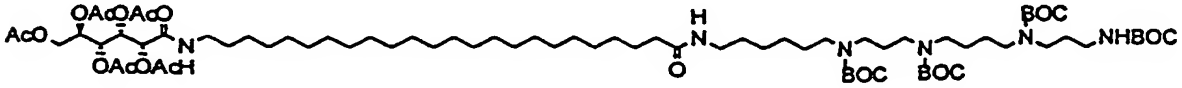
(11) *N*-*t*-Butyloxycarbonyl-*N*-[*t*-butyloxycarbonylaminopropyl(*t*-butyloxycarbonyl)aminobutyl(*t*-butyloxycarbonyl)aminopropyl-1,6-diaminohexane



To 10 (311 mg) in tert-butanol at 40°C was added Pearlman's catalyst (100 mg) and the atmosphere changed to hydrogen. Hydrogenation was left for two days at 40°C, the catalyst filtered off and the solvent removed. The residues were purified by silica chromatography eluting initially with 100:10:0 DCM:MeOH:NH_4OH to bring off the impurities then with 100:10:1 to bring off the desired primary amine as a colourless clear oil, 163 mg, 66%.

$C_{36}H_{71}N_5O_8$ requires 701.5. Found ES+: MH^+ 702.4. δ_H ($CDCl_3$) 1.2-1.8 (52H, m, $(CH_3)_3C$, $H_2NCH_2(CH_2)_4$, CH_2CH_2N), 2.88 (2H, br, CH_2NH_2), 3.0-3.4 (14H, m, CH_2N).

(12) *N*-[*t*-butyloxycarbonylaminopropyl(*t*-butyloxycarbonyl)aminobutyl(*t*-butyloxycarbonyl)aminopropyl(t-butyloxycarbonyl)aminohexyl]-24-(peracetylglucuronylamino)tetracosanamide

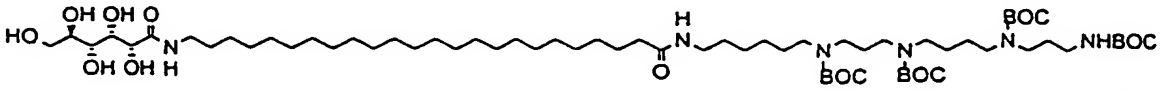


8 (96 mg, 0.125 mmol) in anhydrous dichloromethane (10 cm^3) were added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (48 mg, 0.250 mmol), N-hydroxysuccinimide (22 mg, 0.187 mmol) and the reaction left overnight. To this were then added 11 (92 mg, 0.131 mmol) and triethylamine (63 mg, 0.624 mmol) and the reaction again left overnight. The solvents were removed and the residues purified by silica gradient chromatography eluting with 50-70% ethyl acetate in hexane to yield the product, 157 mg, 86% as a white solid.

δ_H ($CDCl_3$) 1.24 (44H, br, $(CH_2)_{20}CH_2CO$, $N(CH_2)_2(CH_2)_2(CH_2)_2N$), 1.44 (50H, br+m, $(CH_3)_3C$, CH_2CH_2N), 2.04-2.20 (15H, 5xs, CH_3CO), 2.29 (2H, t, CH_2CO), 3.0-3.4 (18H, m, NCH_2), 4.12, 4.31 (2H, m, CH_2OAc), 5.05 (1H, m,

C*H*CH$_2$OAc), 5.29 (1H, d, NHCOC*H*OAc), 5.44, 5.67 (2H, 2xt, AcOCH$_2$CHOAc(C*H*OAc)$_2$), 6.06 (2H, br, NHCO).

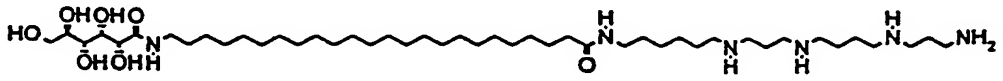
(13) *N*-[*t*-butyloxycarbonylaminopropyl(*t*-butyloxycarbonyl)aminobutyl(*t*-butyloxycarbonyl)aminopropyl(t-butyloxycarbonyl)aminohexyl]-24-(glucuronylamino)tetracosanamide



To 12 (156 mg) dissolved in methanol (10 cm^3) was slowly added with stirring NH_4OH (4 cm^3) until the solution started to become cloudy. After approximately 20 minutes an additional 3 cm^3 of methanol was added to dissolve some of the forming white precipitate. The solution / suspension was stirred for a total of one hour at which point all solvents were removed. The residues were purified by reverse phase chromatography eluting with 2:6:1 CH_2Cl_2:MeOH:NH_4OH to yield a colourless solid, 123 mg, 92%.

$C_{66}H_{128}N_6O_{15}$ requires 1244.9 Found ES$^+$: MNa^+ , 1267.8. δ_H (CD_3OD) 1.28 (44H, br, NH(CH$_2$)$_2$(C*H*$_2$)$_{20}$, N(CH$_2$)$_2$(C*H*$_2$)$_2$(CH$_2$)$_2$N), 1.4-1.9 (50H, brm, (CH$_3$)$_3$C, C*H*$_2$CH$_2$N), 2.16 (2H, t, CH$_2$CO), 3.0-3.4 (18H, m, CH$_2$N), 3.6-3.9 (4H, m, C*H*OH), 4.09, 4.19 (2H, 2xm, C*H*$_2$OH).

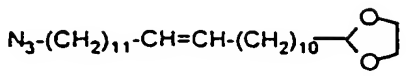
(14) *N*-(aminopropylaminobutylaminopropylaminohexyl)-24-(glucuronylamino)tetracosanamide



13 (120 mg) was treated with 96:4 trifluoroacetic acid : water for 20 minutes and the solvents removed to yield a solid which was taken up into water, filtered through a 0.2μm polypropylene filter and freeze dried to give the product in quantitative yield as a white solid.

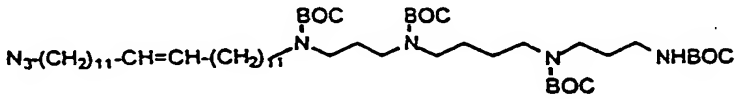
$C_{46}H_{96}N_6O_7$ requires 844.7 Found ES$^+$: MH_2^{2+} , 423.5. δ_H (CD_3OD) 1.28 (44H, m, (C*H*$_2$)$_{20}$CH$_2$CO, N(CH$_2$)$_2$(C*H*$_2$)$_2$(CH$_2$)$_2$N), 1.5-1.8 (6H, m, C*H*$_2$CH$_2$N), 1.81 (4H, m, NCH$_2$(C*H*$_2$)$_2$CH$_2$N), 2.10 (2H, m, CH$_2$CO), 2.16 (4H, m, NCH$_2$C*H*$_2$CH$_2$N), 2.95-3.30 (18H, m, NCH$_2$), 3.55-3.80 (4H, m, C*H*OH), 4.08, 4.20 (2H, 2xm, C*H*$_2$OH).

(15) 23-Azido-1-(1,3-dioxalan-2-yl)tricosa-11-ene



To 23-Chloro-1-(1,3-dioxalan-2-yl)tricosa-11-ene (7.7 g, 16.3 mmol) (synthesised through the Wittig coupling of 12-chlorododecanal and the triphenylphosphonium salt of its acetal using 1 equiv. of LDA in THF at 0°C) in DMF (120 cm^3) was added sodium azide (7.4 g, 113.8 mmol) and the reaction stirred overnight under argon at ambient temperature. The solvent was reduced to approximately 20 cm^3 and water (50 cm^3) added. The solution was extracted with hexane, dried ($MgSO_4$) and the solvent removed to yield a waxy solid 7.023 g, 99%. δ_H ($CDCl_3$) 1.27 (32H, m, $N_3(CH_2)_2(CH_2)_8CH_2CH{=}CHCH_2(CH_2)_8$), 1.60 (4H, m, $CH_2CH_2N_3$, CH_2CHO), 2.00 (4H, m, $CH_2CH{=}$), 3.25 (2H, t, CH_2N_3), 3.83, 3.96 (4H, 2xm, OCH_2), 4.84 (1H, t, CHO), 5.34 (2H, m, CH=CH).

(16) *N*-Azidotetracos-12-enyl(*t*-butyloxycarbonyl)aminopropyl-*N,N'-bis*(*t*-butyloxycarbonyl)-*N'*-*t*-butyloxycarbonylaminopropyl-1,4-diaminobutane

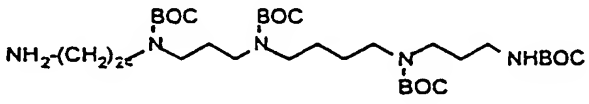


To a rapidly stirring suspension of silica (100 cm^3) and 40% dichloromethane in hexane (100 cm^3) was slowly added tosic acid (1 g) in water (3 cm^3). The suspension was stirred for 10 minutes and used to pack a column. After washing the column with 40% dichloromethane in hexane 15 (537mg) was loaded and slowly eluted over 2 hours with 40% dichloromethane in hexane to give the aldehyde 464 mg, 96% as a colourless oil. To this aldehyde (464 mg, 1.185 mmol) and 9 (655 mg, 1.303 mmol) dissolved in anhydrous THF (40 cm^3) were added Na_2SO_4, acetic acid (20 µl) and sodium triacetoxy borohydride (377 mg, 1.77 mmol) and the reaction left under argon overnight. All solids were filtered off, the solvent removed and the residues taken up into dichloromethane. The solution was washed ($KOH_{(aq)}$), dried ($MgSO_4$), and reduced in volume to approximately 30 cm^3. To the solution was added tert-butyl dicarbonate (388 mg, 1.77 mmol) and the reaction left overnight. The solvent was removed and the residues purified by gradient silica chromatography (20-40% ethyl acetate in hexane) to give the desired product 352 mg, 30% as a colourless gum.

$C_{54}H_{103}N_7O_8$ requires 977.8. Found ES^+: MH^+, 978.7. δ_H ($CDCl_3$) 1.26 (34H, m, $(CH_2)_8CH_2CH{=}CHCH_2(CH_2)_9$), 1.44 (40H, m, $(CH_3)_3C$,

$NCH_2(CH_2)_2CH_2N$), 1.55-1.80 (6H, m, $CH_2CH_2N_3$, $NCH_2CH_2CH_2N$), 2.03 (4H, m, CH_2CH=), 3.05-3.40 (14H, m, NCH_2), 3.24 (2H, t, CH_2N_3), 5.34 (4H, m, CH=CH).

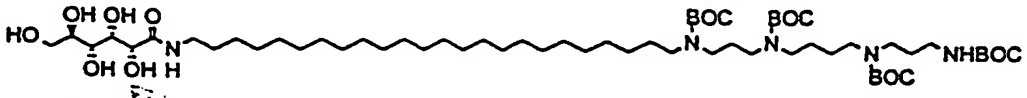
(17) *N*-*t*-Butyloxycarbonyl-*N*-[*t*-butyloxycarbonylaminopropyl(*t*-butyloxycarbonyl)aminobutyl(*t*-butyloxycarbonyl)aminopropyl-1,24-diaminotetracosane



To 16 (204 mg) dissolved in *t*-butanol (20 cm^3) at 40°C was added 10% palladium on carbon (40 mg), and the atmosphere changed to hydrogen. The hydrogenation was maintained at 40°C for 3 days, the catalyst filtered off and the solvent removed. The residues were purified by silica column chromatography eluting with 10% methanol in dichloromethane to elute off impurities followed by 90:10:1 DCM:MeOH:NH_4OH to bring off the desired amine as a colourless gum 61mg, 31%.

$C_{54}H_{107}N_5O_8$ requires 953.8. Found ES^+: MH^+ , 954.6. δ_H ($CDCl_3$) 1.24 (42H, br, $(CH_2)_{21}CH_2NH_2$), 1.43 (42H, br, $(CH_3)_3C$, $NCH_2(CH_2)_2CH_2N$, $CH_2(CH_2)_{22}NH_2$), 1.70 (4H, m, $NCH_2CH_2CH_2N$), 2.70 (2H, t, CH_2NH_2), 3.0-3.4 (14H, brm, CH_2N), 5.35 (1H, b r, CONH).

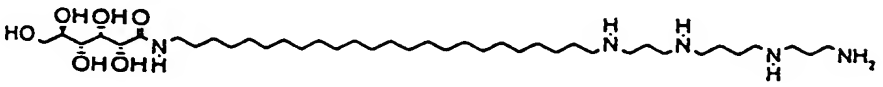
(18) *N*-*t*-Butyloxycarbonyl-*N*-[*t*-butyloxycarbonylaminopropyl(*t*-butyloxycarbonyl)aminobutyl(*t*-butyloxycarbonyl)aminopropyl-*N'*-glucuronyl-1,24-diaminotetracosane



To 17 (62 mg, 0.065 mmol) in anhydrous methanol (8 cm^3) were added δ-gluconolactone (17 mg, 0.097 mmol) and triethylamine (26 mg, 0.260 mmol) and the reaction stirred overnight at ambient temperature under argon. The solvents were removed and the residues purified by reverse phase chromatography eluting with 2:6:1 DCM:MeOH:H_2O to give the product, 32 mg, 43% as a white solid.

$C_{60}H_{117}N_5O_{14}$ requires 1131.9 Found ES^+: MH^+ , 1132.8. δ_H ($CDCl_3$) 1.25 (40H, br, $N(CH_2)_2(CH_2)_{20}$), 1.44 (40H, br, $(CH_3)_3C$, CH_2CH_2CO, $NCH_2CH_2(CH_2)_{20}CH_2CH_2N$), 1.6-1.8 (8H, m, $NCH_2CH_2CH_2N$, $NCH_2(CH_2)_2CH_2N$), 3.0-3.4 (16H, brm, NCH_2), 3.80 (4H, br, CHOH), 4.1, 4.3 (2H, 2xbr, CH_2OH), 5.37 (1H, br, $NHCO_2$), 7.22 (1H, br, NHCO).

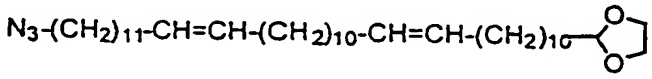
(19) *N*-Aminopr pylaminobutylaminopropyl-*N'*-glucuronyl-1,24-diaminotetracosane



18 (32 mg) was treated as for the synthesis of 14 to give the product in quantitative yield as a white solid.

$C_{40}H_{85}N_5O_6$ requires 731.7 Found ES$^+$: MH^+ , 732.6, MH_2^{2+} , 367.0. δ_H (CD_3OD) 1.28 (40H, br, $NH(CH_2)_2(CH_2)_{20}$), 1.53 (2H, p, $CONHCH_2CH_2$), 1.69 (2H, p, $(CH_2)_{22}CH_2CH_2NHCH_2$), 1.81 (4H, m, $NCH_2(CH_2)_2CH_2N$), 2.10 (4H, m, $NCH_2CH_2CH_2N$), 2.9-3.3 (16H, m, CH_2N), 3.55-3.85 (4H, m, CHOH), 4.09, 4.20 (2H, 2xm, CH_2OH).

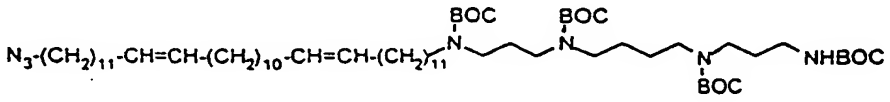
(20) 35-Azido-1-(1,3-dioxalan-2-yl)pentatriaconta-11,23-diene



To 35-Chloro-1-(1,3-dioxalan-2-yl)pentatriaconta-11,23-diene (1.602 g, 2.690 mmol) (synthesised through the Wittig coupling of 12-chlorododecanal and the triphenylphosphonium salt of 23-Chloro-1-(1,3-dioxalan-2-yl)tricosa-11-ene using 1 equiv. of LDA in THF at 0°C) in anhydrous DMF (70 cm^3) was added sodium azide (1.224 g, 18.83 mmol) and the reaction heated for 5 days at 50°C under argon. The solvent was reduced to almost dryness and the residues taken up into water (150 cm^3) and ethyl acetate (150 cm^3). The aqueous layer was extracted with ethyl acetate (4 x 150 cm^3), the fractions combined, washed (2 x 150 cm^3 water), dried ($MgSO_4$) and the solvent removed to quantitatively yield the azide as a pale yellow waxy solid.

$C_{38}H_{71}N_3O_2$ requires 601.6. Found ES$^+$: MNH_4^+ , 619.6. δ_H ($CDCl_3$) 1.27 (48H, br, $N_3(CH_2)_2(CH_2)_8CH_2CH=CHCH_2(CH_2)_8CH_2CH=CHCH_2(CH_2)_8$), 1.61 (4H, m, CH_2CH, $CH_2CH_2N_3$), 2.00 (8H, m, $CH_2CH=$), 3.25 (2H, t, CH_2N_3),. 3.8-4.05 (4H, m, CH_2O), 4.84 (1H, t, CH), 5.34 (4H, m, CH=CH).

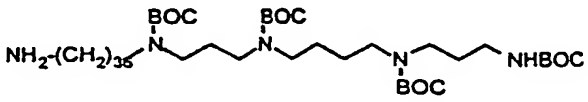
(21) *N*-Azidohexatriaconta-12,24-dienyl (*t*-butyloxycarbonyl) aminopropyl-*N,N'-bis* (*t*-butyloxycarbonyl)-*N'*-*t*-butyloxycarbonylaminopropyl-1,4-diaminobutane



To a rapidly stirring suspension of silica (200 cm^3) and 50% dichloromethane in hexane (200 cm^3) was slowly added tosic acid (2 g) in water (6 cm^3). The suspension was stirred for 10 minutes and used to pack a column. After washing the column with 50% dichloromethane in hexane 20 (998 mg) was loaded and eluted over 2 hours with 50% dichloromethane in hexane to give the aldehyde 591 mg, 65% as a colourless oil. To this aldehyde (591 mg, 1.059 mmol) and 9 (559 mg, 1.112 mmol) dissolved in anhydrous 30:70 MeOH:DCM (30 cm^3) was added Na_2SO_4 and the reaction heated at reflux overnight. Sodium triacetoxy borohydride (449 mg, 2.118 mmol) was added and the reaction left for 4 hours. The drying agent was removed, water (1 cm^3) added, and the reaction left overnight. t-Butyl dicarbonate (694 mg, 3.178 mmol) and triethylamine (536 mg, 5.296 mmol) were added and the reaction again left overnight. The solvent was removed and the residues purified by gradient silica chromatography eluting with 20-40% ethyl acetate in hexane to yield the product 226 mg, 19% as a colourless gum.

$C_{66}H_{125}N_7O_8$ requires 1144.0. Found ES^+: MNa^+ , 1166.8. δ_H ($CDCl_3$) 1.27 (50H, br, $N_3CH_2(CH_2)_9CH_2CH{=}CHCH_2(CH_2)_8CH_2CH{=}CHCH_2(CH_2)_8$), 1.4-1.9 (46H, m, $(CH_3)_3C$, CH_2CH_2N), 2.0 (8H, m, $CH_2CH{=}$), 3.05-3.4 (14H, m, CH_2N), 3.24 (2H, t, CH_2N_3), 5.34 (4H, m, CH=).

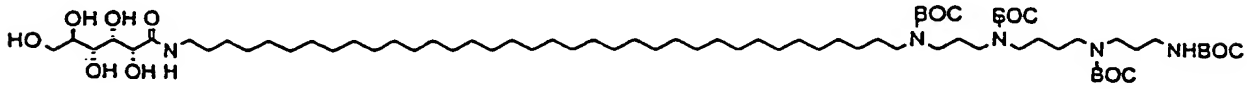
(22) *N*-*t*-Butyloxycarbonyl-*N*-[*t*-butyloxycarbonylaminopropyl(*t*-butyloxycarbonyl)aminobutyl(*t*-butyloxycarbonyl)aminopropyl]-1,36-diaminohexatriacontane



To **21** (220 mg) dissolved in tert-butanol at 40°C was added 10% palladium on carbon (60 mg) and the atmosphere changed to hydrogen. The hydrogenation was left for two days, the catalyst filtered off, the solvent removed and the residues purified by silica chromatography eluting with 100:10 dichloromethane : methanol then with 100:10:1 dichloromethane : methanol : NH_4OH to give the product as a colourless gum 147 mg, 68%.

$C_{66}H_{131}N_5O_8$ requires 1122.0 Found ES$^+$: MH^+, 1122.9. δ_H ($CDCl_3$) 1.25 (64H, br s, $(CH_2)_{32}(CH_2)_2NH_2$), 1.4-1.8 (48H, br s, $(CH_3)_3C$, CH_2CH_2N), 2.88 (2H, t, CH_2NH_2), 3.0-3.4 (14H, br, CH_2N).

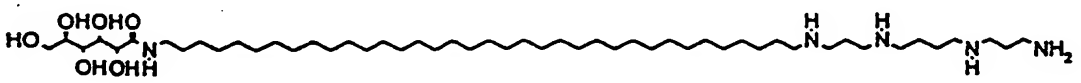
(23) *N*-*t*-Butyloxycarbonyl-*N*-[*t*-butyloxycarbonylaminopropyl(*t*-butyloxycarbonyl)aminobutyl(*t*-butyloxycarbonyl)aminopropyl]-*N'*-glucuronyl-1,36-diaminohexatriacontane



To 22 (130 mg, 0.116 mmol) in anhydrous methanol (8 cm^3) under argon at 40°C were added triethylamine (47 mg, 0.463 mmol) and δ-gluconolactone (62 mg, 0.347 mmol) and the reaction left overnight. The solvent was removed and the residues purified by gradient reverse phase silica chromatography eluting with 2:6:1 to 2:6:0.25 dichloromethane : methanol : water to give the product as a colourless solid 83 mg, 55%.

$C_{72}H_{141}N_5O_{14}$ requires 1300.0 Found ES$^+$: MNa^+, 1323.0. δ_H ($CDCl_3$) 1.25 (64H, br, $N(CH_2)_2(CH_2)_{32}$), 1.35-1.80 (48H, br m, CH_2CH_2N, $(CH_3)_3C$), 3.0-3.4 (16H, br m, NCH_2), 3.55-4.0 (4H, br, CHOH), 4.20, 4.45 (2H, 2xbr, CH_2OH), 5.28 (1H, br, $NHCO_2$), 7.78 (1H, br, $CONH(CH_2)_{36}$).

(24) *N*-aminopropylaminobutylaminopropyl-*N'*-glucuronyl-1,36-diaminohexatriacontane



23 (83 mg) was treated as in the synthesis of 14 to quantitatively yield the product as a white solid.

$C_{52}H_{1091}N_5O_6$ requires 899.8 Found ES$^+$: MH^+, 900.9. δ_H (CD_3OD) 0.8-2.2 (76H, br, $(CH_2)_{32}(CH_2)_2N$, CH_2CH_2N), 2.8-3.4 (16H, br, CH_2N), 3.5-3.9 (4H, br, CHOH), 4.08, 4.20 (2H, 2xbr, CH_2OH).

The advantageous properties of the lipids according to the invention may be demonstrated in the following tests:

PRONASE and DNase treatment

One of the ultimate goals of current gene therapy research is to develop a delivery system which will remain stable and effective *in vivo*, since this would remove the need for expensive and time-consuming ex-*vivo* manipulations. Since intravenous administration offers the possibility of delivery to the largest number of tissue sites, survival of the gene delivery complex in the presence of serum could be an important feature of any effective technology. Many published studies have demonstated the susceptibility of gene delivery complexes to inactivation by serum even at levels as low as 10%. This effect is due, at least in part, to the destabilisation of complexes by a poorly understood mechanism, and this can lead to degradation of the DNA within the complex by serum-associated nucleases. We have therefore formed complexes between the lipids of the invention and plasmid DNA and subjected these to treatment with purified DNase and pronase as well as with 50% foetal calf serum. Integrity of the plasmid DNA was then measured.

Methods

Plasmid DNA (pEG*lacZ*) was prepared at a concentration of 120μg/ml in water. A lipid according to the invention [for example the hexamine H18 described in the Example above] was prepared in a range of concentrations such that charge ratios of lipid:DNA of 0.25:1, 1:1, 2:1, 4:1, 8:1 would be obtained (based on the assumption that DNA at 120 μg/ml is equivalent to 0.387mM of negative charge, and the hexamine H18 for example at 10mg/ml is equivalent to 36.23mM of positive charge). An equal volume of DNA was added dropwise to a vortexing tube containing the lipid in water. For DNase treatment, DNase I (FPLCpure, Pharmacia) was added at a concentration of 1 unit/1μg of DNA and tubes were incubated at 37°C for 10 minutes. To inhibit further action of DNase, EGTA was added to a final concentration of 25mM. For pronase treatment, protease XIV (Sigma) was added to samples to a final concentration of 150 μg/ml, and samples incubated for 30 min at 37°C.

Complexes were disrupted in 0.5% SDS with incubation at 55°C for 20 min. Serum treatment involved incubating the samples in the presence of 50% foetal calf serum (final concentration) for 30 min at 37°C. EGTA was added to a final concentration of 50mM in an attempt to prevent subsequent action of serum-associated nucleases. Finally, all samples were analysed by electrophoresis on 0.8% agarose gels.

Results

Analysis of the mobility of plasmid DNA through gels demonstrated that, as the amount of lipid increased. the DNA tended to be retarded in the wells. Thus, for example, at a charge ratio of 2:1 (H18:DNA), no DNA entered the gel, and at higher ratios, the plasmid DNA was no longer visible by ethidium bromide fluorescence (see below), suggesting that the DNA had become fully condensed. The H18/DNA condensates were resistant to treatment by pronase. In addition, DNA condensed with H18 at a charge ratio of at least 2:1 was resistant to treatment by DNase. At charge ratios of 2:1 or greater, addition of serum to 50% did not lead to an increase in the amount of DNA degradation, suggesting that lipids according to the invention are stable in serum.

PHYSICAL CHEMICAL ASSAYS

Two physical chemical assays can be used to assess the ability of the lipids of the invention to compact supercoiled DNA and to determine the stability of the condensed particles.

Assay 1

The first assay involves the use of ethidium bromide, a molecule which fluoresces when intercalated into the DNA helix. Solutions containing DNA and a lipid according to the invention are prepared so that the charge ratio between the negatively charged phosphate groups of the DNA and the postively charged polyamines of the lipids varies between zero and three [see the "Methods" in the previous section]. After ethidium bromide has been added to each solution the fluorescence reading is measured. As the charge ratio increases towards charge neutrality, because of the increasing amounts of lipid present, there is a progressive decrease in the

fluorescence of the ethidium bromide when this molecule is excluded from binding to the DNA as compaction occurs. The point at which compaction is complete corresponds to the point at which the fluorescence reading levels-off at a minimum. In the case of thelipids of the invention the fluorescence minimum is reached at charge ratios of lipid to DNA in the range of 0.8 to 2.5. This assay has been used to demonstrate that lipid are compaction competent under conditions of physiological salt (150mM NaCl) and at acidic conditions down to pH 3.0. Repeating the assay also shows that the compacted DNA particles are stable for many hours both in physiological salt and under low pH conditions.

Assay 2

The second assay involves gel electrophoresis using ethidium bromide as a stain. Samples of lipid and DNA are prepared as before and loaded into separate lanes in a polyacrylamide gel. After electrophoresis the fluorescence reading of each lane is determined. Two effects are observed. First, as the charge ratio increases towards neutrality the distance that the DNA/lipid complex travels through the gel decreases progressitvely. This is a result of two physical processes; compaction, which renders the DNA less able to move through the viscous gel and neutralisation of DNA negative charge, which reduces the electrostatic attraction between the complex and the cathode. Second, as the charge ratio increases beyond neutrality the brilliance of the fluorescent response decreases as the ethidium bromide stain is excluded from the DNA helix. This assay has been used to confirm that the lipids according to the invention cause DNA compaction close to the point of charge neutrality, in agreement with theory.

TRANSFECTION OF MAMMALIAN CELLS WITH LIPID CONDENSED DNA COMPLEXES

Condensation of DNA

Plasmid DNA (pEG*lacZ*) was prepared at concentrations of typically 60 or 120 µg/ml in water. Solutions of lipids according to the invention were prepared in water over a range of concentrations (typically 30 to 960 µg/ml). An equal volume of DNA was added dropwise to a tube containing a solution of the lipids whilst vortexing the tube.

CHO Transfection protocol

Chinese Hamster Ovary (CHO) cells were seeded in to 24 well plates at 100,000 cells per well 24h before experiment. Cells were washed once in Optimem™ medium prior to transfection. Wash medium was removed and replaced with 0.5ml of Optimem™ to which the required amount of lipid condensed DNA was added (typically 1 to 5 μg DNA equivalent). Usually three replicate transfection wells were set up per condensed DNA sample tested. Cells were incubated for a further 3-4 h at 37°C, 5% CO_2 before removal of the complex and additon of 1ml of fresh medium (Iscoves™ medium: modified DMEM plus glutamate, asparagine, adenosine, guanosine, cytidine, uridine, thymidine and 10% dialysed foetal calf serum). Cells were cultured for a further 48-72 hours before harvesting and assay. Levels of Beta galactosidase reporter gene activity were determined using an enzyme assay system from Promega as follows. Cells were washed twice with 1ml of phosphate buffered saline and solubilised in 200ul of 1 x cell lysis buffer. 50ul of cell extract was incubated with the provided buffer and substrate o-nitrophenyl-β-D-galactopyranoside and the optical density measured spectrophotometrically. Levels of β-gal expression were quantitated by reference to the standard curve and related to the amount of protein in the extract (measured using the BCA assay kit from Pierce) to give a final value expressed as mU of β–gal per mg of protein.

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CLAIMS

1. A bipolar lipid comprising a cationic head (1) a hydrophobic backbone (2) and a hydrophilic tail (3) in which:
   (A) the cationic head comprises two or more cationic centres, each centre containing at least one heteroatom and being covalently linked to one or more other centres by one or more carbon containing spacer groups;
   (B) the hydrophobic backbone comprises one or more hydrocarbon chains; and
   (C) the hydrophilic tail comprises one or more acyclic hydrophilic hydrocarbons each containing two or more atoms or groups capable of being solvated by water;
   each of said components (1) to (3) being covalently linked head (1) to backbone (2) to tail (3) and arranged such that at least one hydrocarbon chain in the hydrophobic backbone (2) is covalently linked to a heteroatom in a cationic head (1) and each hydrophilic hydrocarbon in the hydrophilic tail (3) is covalently linked to a chain in the backbone (2) to achieve at least a ten atom spacing along the chain between the tail (3) and the head (1).

2. A lipid according to Claim 1 wherein each cationic centre is an amino group.

3. A lipid according to Claim 1 or Claim 2 wherein the number of cationic centres in the cationic head is from three to six.

4. A lipid according to any one of Claim 1 to Claim 3 wherein each carbon containing spacer group is an optionally substituted aliphatic, cycloaliphatic, heteroaliphatic, heterocycloaliphatic, aromatic or heteroaromatic group.

5. A lipid according to any one of Claim 1 to Claim 4 wherein each hydrocarbon chain in the hydrophobic backbone is an optionally substituted straight or branched aliphatic or heteroaliphatic chain containing from ten to around one hundred chain-linked atoms.

6. A lipid according to Claim 5 wherein the hydrophobic backbone has one or two hydrocarbon chains indirectly linked through a linker atom or group to a carbon atom in a spacer group connecting two cationic centres in the cationic head (1).

7. A lipid according to any of Claim 1 to Claim 5 wherein each hydrophilic hydrocarbon in the hydrophilic tail (3) is attached to a hydrocarbon chain of the hydrocarbon backbone (2) at the terminal carbon atom of said chain distal to the chain carbon atom attached to the cationic head (1).

8. A lipid according to any of the preceding Claims which has the formula (1):

$$[R^1]_m\text{-}(L^1)_n\text{-}R^2 \qquad (1)$$

wherein R^1 is a hydrocarbon chain optionally substituted by one or more acyclic hydrophilic hydrocarbons each containing two or more atoms or groups capable of being solvated by water, provided that at least one hydrocarbon chain is substituted by at least one hydrophilic hydrocarbon and each hydrophilic hydrocarbon is attached to the hydrocarbon chain to achieve at least a ten atom spacing along the chain between the hydrophilic hydrocarbon and the group $-(L^1)_n-R^2$;
m is an integer from 1 to 6;
L^1 is a linker atom or group;
n is zero or the integer 1;
R^2 is an optionally substituted aliphatic, cycloaliphatic, heteroaliphatic, heterocycloaliphatic, aromatic or heteroaromatic group containing two or more cationic centres, and provided that each $[R^1]_m\text{-}(L^1)_n$- group is attached to a heteroatom in a cationic centre in R^2;
and the salts, solvates and hydrates thereof.

9. A lipid according to Claim 8 which has the formula (1a):

$$[R^7]_p\text{-}(L^3)_q\text{-}[R^6]_m\text{-}(L^1)_n\text{-}R^2 \qquad (1a)$$

wherein R^2, L^1, m and n are as defined for formula (1);
R^6 is a hydrocarbon chain;
L^3 is a linker atom or group;
R^7 is a hydrophilic hydrocarbon containing two or more atoms or groups capable of being solvated by water;
q is zero or an integer from one to six;
p is an integer from one to six;
and the salts, solvates and hydrates thereof, provided that each R^7 or L^3 group, when present, is attached to a group R^6 to achieve at least a ten atom spacing along R^6 between R^7 or L^3 and the group $-(L^1)_n-R^2$.

10. A lipid according to Claim 9 wherein R^2 is a hydrogen atom and R^3 and R^4 is each a group $Sp^1[WSp^2]_bWSp^3$ or $-Sp^1[WSp^2]_bWH$ in which Sp^1, Sp^2 and Sp^3, which may be the same or different, is each a spacer group, W is a cationic centre and b is zero or an integer from one to six.

11. A lipid according to Claim 10 where Sp^1, Sp^2 and Sp^3 is each an optionally substituted aliphatic, cycloaliphatic, aromatic or heteroaromatic group.

12. A lipid according to Claim 11 wherein Sp^1, Sp^2 and Sp^3 is each an optionally substituted C_{1-6}alkylene chain.

13. A lipid according to any one of Claim 9 to Claim 12 wherein W is a -NH- group.

14. A lipid according to any one of Claim 9 to Claim 13 wherein b is an integer from 2 to 4.

15. A lipid according to Claim 9 wherein the group R^2 is a group $-NH[Sp^1NHSp^2]NH_2$, $-NH[Sp^1NHSp^2NHSp^2]NH_2$ or $-NH[Sp^1NHSp^2NHSp^2]NHCH_3$ wherein Sp^1 is $-CH_2-$ and each Sp^2 is $-(CH_2)_3-$ or $-(CH_2)_4-$.

16. A lipid according to any one of Claim 9 to Claim 15 wherein n in $-(L^1)_n-$ is the integer 1.

17. A lipid according to Claim 16 wherein L^1 is a group $-X^1Alk^2-$ or $-[X^1]_2Alk^1X^1Alk^2-$ in which X^1 is an -O- or -S- atom or a -C(O)-, -C(O)O-, -C(S)-, -S(O), $-S(O)_2-$ $-N(R^5)-$, [where R^5 is a hydrogen atom, straight or branched alkyl group such as a methyl or ethyl group or an $-Alk^1X^1-$ chain], $-CON(R^5)-$, $-OC(O)N(R^5)-$, $-CSN(R^5)-$, $-N(R^5)CO-$, $N(R^5)C(O)O-$, $-N(R^5)CS-$, $-S(O)N(R^5)-$, $-S(O)_2N(R^5)-$, $-N(R^5)S(O)-$, $-N(R^5)S(O)_2-$, $-N(R^5)CON(R^5)-$, or $-N(R^5)SO_2N(R^5)-$ group [where any of these groups contains two R^5 substituents these may be the same or different]; and Alk^1 and Alk^2 which may be the same or different is each an optionally substituted straight or branched C_{1-6}alkylene, C_{2-6}alkenylene or C_{2-6}alkynylene chain optionally interrupted or terminated by one or more, e.g. one, two or three, carbocyclic or heterocarbocyclic groups and/or heteroatoms or heteroatom containing groups X^1 as just defined.

18. A lipid according to Claim 17 wherein X^1 is a -CONH- group, Alk^1 is a $-CH_2-CH<$ chain and Alk^2 is a $-(CH_2)_4-$, $-(CH_2)_5-$ or $-(CH_2)_6-$ chain.

19. A lipid according to any one of Claim 9 to Claim 18 wherein m is an integer 1 or 2.

20. A lipid according to any one of Claim 9 to Claim 19 wherein R^6 is an optionally substituted C_{10-60}aliphatic chain.

21. A lipid according to Claim 20 wherein R^6 is a linear, optionally substituted C_{16-38}alkylene chain.

22. A lipid according to any one of Claim 9 to Claim 21 wherein q is the integer 1 and p is the integer 1 or 2.

23. A lipid according to any one of Claim 9 to Claim 22 wherein L^3 is an atom or group $-X^1-$, $-X^1Alk^1X^1-$ or $[X^1Alk^1]_1X^1Alk^2X^1$ in which X^1 is

an -O- or -S- atom or a -C(O)-, -C(O)O-, -C(S)-, -S(O), -$S(O)_2$- -$N(R^5)$-, [where R^5 is a hydrogen atom, straight or branched alkyl group such as a methyl or ethyl group or an -Alk^1X^1- chain], -$CON(R^5)$-, -$OC(O)N(R^5)$-, -$CSN(R^5)$-, -$N(R^5)CO$-, $N(R^5)C(O)O$-, -$N(R^5)CS$-, -$S(O)N(R^5)$-, -$S(O)_2N(R^5)$-, -$N(R^5)S(O)$-, -$N(R^5)S(O)_2$-, -$N(R^5)CON(R^5)$-, or -$N(R^5)SO_2N(R^5)$- group [where any of these groups contains two R^5 substituents these may be the same or different]; and Alk^1 and Alk^2 which may be the same or different is each an optionally substituted straight or branched C_{1-6}alkylene, C_{2-6}alkenylene or C_{2-6}alkynylene chain optionally interrupted or terminated by one or more, e.g. one, two or three, carbocyclic or heterocarbocyclic groups and/or heteroatoms or heteroatom containing groups X^1 as just defined.

24. A lipid according to Claim 23 wherein L^3 is a -NHCO-, -CONH-, -$CONH(CH_2)_2NHCO$-, or -$[CONH(CH_2)_2-]_2NCO(CH_2)_2CONH$ group.

25. A lipid according to any one of Claim 9 to Claim 24 wherein R^7 is a synthetic or naturally occurring polyol or a poly(alkylene oxide) or a derivative thereof.

26. A lipid according to Claim 25 wherein R^7 is a poly(alkylene oxide) or a derivative thereof.

27. A lipid according to Claim 26 wherein R^7 is a poly(ethylene oxide).

28. A lipid according to any one of the Examples herein.

29. A targeted lipid according to any one of Claim 1 to Claim 28 in which the bipolar lipid is assembled with one or more targeting molecules.

30. A lipid according to Claim 29 wherein each targeting molecule is an antibody or a fragment or a derivative thereof.

31. A lipid complex comprising a bipolar lipid according to any one of Claim 1 to Claim 28 in association with one or more bioactive substances.

32. A complex according to Claim 31 wherein each bioactive substance is a bioactive protein, peptide, polysaccharide, nucleic acid, oligonucleotide or a derivative thereof, lipid, glycolipid, lipoprotein, lipopolysaccharide or viral, bacterial, protozoal, cellular or tissue fraction.

33. A complex according to Claim 32 wherein the bioactive substance is a polyanion.

34. A complex according to Claim 33 wherein the bioactive substance is a nucleic acid.

35. A complex according to any one of Claims 31 to 34 containing two or more different bipolar lipids.

36. A complex according to Claim 35 wherein one bipolar lipid has a hydrophilic tail formed by a poly(alkylene oxide) or a derivative thereof and each of the others has a hydrophilic tail formed by a synthetic or naturally occurring polyol.

37. A complex according to Claim 36 wherein the poly(alkylene oxide) is poly(ethylene oxide).

38. A complex according to any one of Claim 31 to Claim 37 additionally comprising one or more targeting molecules or a targeted lipid of Claim 29.

39. A composition comprising a complex according to any one of Claim 31 to Claim 38 and one or more other lipids.

40. A composition according to Claim 39 wherein each other lipid is a neutral or cationic lipid.

41. A composition comprising a complex according to any one of Claim 31 to Claim 40 and one or more pharmaceutically acceptable carriers, excipients or diluents.

42. A method of preparing a lipid as claimed in any one of Claims 1 to 28, comprising coupling:

(A) a cationic head comprising two or more cationic centres, each centre containing at least one heteroatom and being covalently linked to one or more others by one or more carbon containing spacer groups;

(B) a hydrophobic backbone comprising one or more hydrocarbon chains; and

(C) a hydrophilic tail comprising one or more hydrophilic hydrocarbons each containing two or more atoms or groups capable or being solvated by water, wherein starting materials A), B) and C) contain one or more reactive functional groups suitable for facilitating coupling.

43. A method of preparing a lipid as claimed in any one of Claims 1 to 28, comprising the step of deprotecting a protected derivative of said lipid.

44. A method of preparing a complex as claimed in any one of Claims 31 to 38 comprising mixing a lipid as claimed in any one of claims 1 to 30 with a bioactive substance.

45. Use of a complex as claimed in any one of Claims 31 to 38 for delivering a bioactive substance to cells *in vitro*.

46. A composition as claimed in any one of Claims 39 to 41 for use in delivering a bioactive substance to cells *in vivo*.

47. A composition as claimed in any one of Claims 39 to 41 for use as a medicament.

48. Use of a composition as claimed in any one of claims 39 to 41 for the preparation of a medicament for the delivery of a bioactive substance, preferably a therapeutic, diagnostic or immunomodulatory agent.

49. Method of delivering a bioactive substance to a human or non-human animal wherein said bioactive substance, preferably a therapeutic, diagnostic or immunomodulatory agent, is administered in the form of a complex as defined in any one of claims 31 to 38.

INTERNATIONAL SEARCH REPORT

Internat al Application No
PCT/GB 00/01613

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07C237/22 A61K47/48 A61K9/127 C12N15/88

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07C A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal, CHEM ABS Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	FUHRHOP J -H ET AL: "BOLAAMPHIPHILES WITH MANNOSE- AND TETRAALKYLAMMONIUM HEAD GROUPS ASCOATINGS FOR NUCLEIC ACIDS AND POSSIBLE REAGENTS FOR TRANSFECTIONS" CHEMISTRY AND PHYSICS OF LIPIDS,IR,LIMERICK, vol. 43, 1 April 1987 (1987-04-01), pages 193-213, XP000562618 ISSN: 0009-3084 page 193 -page 201	1-49

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 July 2000	13/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Zervas, B

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	WO 96 17823 A (RHONE-POULENC RORER) 13 June 1996 (1996-06-13) cited in the application claims; examples	1,29,31, 39,45, 46,49

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Information on patent family members

Interna[illegible]cation No
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